Macrophages are professional phagocytes involved in the developmental and homeostatic clearance of apoptotic cells and the detection and engulfment of pathogens. We have investigated such behaviour in Drosophila embryonic hemocytes (macrophages), allowing the adoption of powerful genetics and live, in vivo imaging within the fly embryo.

During their initial dispersal around the embryo, these macrophages detect and clear apoptotic cells, which can be visualised live by fluorescent annexin V injection. Apoptosis of individual epithelial cells can also be triggered by UV-irradiation, leading to rapid macrophage-mediated engulfment. We have combined this with far-red annexin V injection to visualise this process in three colours and have generated 3D rendered movies of efferocytosis.

We have found that macrophages utilise two, morphologically and molecularly distinct modes of phagocytosis. Macrophages use Arp2/3 complex-dependent lamellipods to chemotax towards and envelope cellular debris. Alternatively, they can extend Arp2/3 complex-independent, long, thin, grasping filopods to draw material back into the cell body. The latter restricts the size of particle that can be engulfed, limiting Arp2/3-deficient macrophages to ‘nibbling’ at apoptotic corpses.

‘Lamellipodial phagocytosis’ appears to be the primary mode of engulfment utilised by these highly motile macrophages. However, we can promote ‘filopodial phagocytosis’ by either suppressing motility, by spatially constricting the macrophages or by triggering an inflammatory response through laser-induced wounding of the embryo epithelium. In the latter case, recruited macrophages will extend a filopod to engulf out of reach debris rather than leave the wound.

From these results we conclude that lamellipodial phagocytosis is Arp2/3 complex and motility dependent whereas filopodial phagocytosis is Arp2/3 complex and motility independent, allowing macrophages to reach apoptotic debris even when they are unable to chemotax towards it. Ultimately, this cytoskeletal robustness confers phagocytic plasticity ensuring macrophages can clear apoptotic debris under all circumstances.
P.02 Intestinal permeability – a new target for arthritis?

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The presence of subclinical gut inflammation has been associated with severity of joint inflammation in certain spondyloarthropathies, however, the role of gut inflammation in rheumatoid arthritis (RA) remains speculative. Recently, serum levels of lipopolysaccharide (LPS) binding protein (LBP), an acute phase protein produced by the gut and liver in response to LPS entering the blood stream from the gut, has been reported to be a sensitive biomarker for RA severity. Although LBP may simply reflect systemic inflammation, increases in serum LBP may also be a sensitive measure of increased gut permeability.

Using measurements of LBP and intestinal fatty acid binding protein (iFABP) concentrations in serum we confirmed that patients with RA had an increase in intestinal permeability compared to healthy controls. To further investigate the effects of altered intestinal permeability on arthritic disease, and to allow us to directly manipulate intestinal permeability, we moved to the antigen-induced arthritis mouse model. We report histopathological signs of subclinical inflammation and an increased epithelial permeability in the intestines of mice with arthritis. The histopathological changes were mirrored by an increased production of pro-inflammatory cytokines including TNF-alpha and IL-23 released by intestinal immune cells as well as a decrease in the anti-inflammatory IL-10. Furthermore, Claudin-8/-/- mice, which have constitutively increased intestinal permeability, demonstrated more severe joint inflammation compared to wild-type mice indicating a direct relationship between intestinal permeability and arthritis. Of interest, treatment of mice with AT-1001, a zonulin antagonist that maintains gut epithelial integrity, resulted in a significant reduction in joint inflammation.

In conclusion, we confirm the presence of increased intestinal permeability in arthritis and we report that direct manipulation of the permeability of the gut epithelium can alter the severity of arthritis in mice and that targeting intestinal tight junction stability may be a novel clinical intervention for patients with RA.

P.03 Investigating the role of regenerative medicine therapies in modulating immune cells in renal ischaemia reperfusion injury

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Ischaemia-reperfusion injury (IRI), the most common cause of acute kidney injury (AKI), is characterised by two phases: an initial insult mediated by immune cell infiltration into the kidney, and a secondary response which brings about the resolution of AKI. Immune cells mediate both injury and repair in the post-ischaemic kidney; M1 pro-inflammatory macrophages are the main perpetrators of AKI, whereas their polarisation towards an M2 anti-inflammatory and reparative phenotype promotes AKI resolution.

We have previously shown in a preclinical model that cell-based regenerative therapies are able to ameliorate AKI, possibly via the release of paracrine factors. Preliminary data from our group suggest that cell therapies improve renal function and promote tubular regeneration in a rodent model of
AKI. Cell therapies administered through IV injection were sequestered in the lung, and underwent apoptosis within 48h. We showed that these apoptotic cells were engulfed by CD68+ macrophages, which released the anti-inflammatory cytokine IL-10. IL-10 is known to dampen immune responses and improve AKI, while efferocytosis is both an anti-inflammatory and anti-immunogenic process that stimulates immune cells towards an anti-inflammatory phenotype that could further improve the outcome of AKI.

We are currently investigating how these processes are regulated, by analysing in the first instance the temporal profile of macrophage polarisation in response to cell administration. We are also determining the role of T-cells, specifically regulatory T-cells, in the amelioration response to cell therapies in mice after AKI. Understanding the cellular and molecular mechanisms through which the immune system responds to the cell therapies and provides protection or repair in a preclinical model of AKI will allow us to develop regenerative medicine therapies with potential for clinical applications.

P.04 IL-1β processing is tightly regulated by Gata6-prostacyclin-IL-10-dependant axis in tissue-resident macrophages

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The pro-inflammatory cytokine IL-1β is widely recognized as an alarm signal, rapidly produced and released by cells under major stress condition. Despite its tremendous role controlling the local and systemic activation of the immune system, the regulation of IL-1β production is still poorly understood. The generally accepted process is that the production of the proform (pro-IL-1β) follows the recognition of Pathogen Associated Molecular Patterns (PAMPs) and its conversion into its active form (mature IL-1β) requires activation of the inflammasome by a second signal. However, little is know concerning its negative regulation and the controls in place to avoid its unwanted processing and release in absence of secondary signal.

Gata6 has been identified as a major factor in the control and development of tissue-resident peritoneal macrophages (pMΦ) in mice and provides a unique model to understand how tissue-specific specialisation of phenotype controls inflammatory responses. Our recent investigation shows that Gata6-KO pMΦ have a marked dysregulation of IL-1β secretion. In contrast to wild type (WT) pMΦ, LPS stimulation alone is sufficient to induce active secretion of IL-1β in a time- and concentration-dependant manner by Gata6-KO pMΦ.

Using Gata6-KO pMΦ as a tool to investigate the control of IL-1β processing by WT pMΦ, we identified the Gata6-prostacyclin-IL-10-dependant inhibitory pathway in WT pMΦ, actively controlling the processing of pro-IL-1β in the absence of secondary signal.

Our data show for the first time that the processing of IL-1β following recognition of PAMPs is actively inhibited by prostacyclin-controlled IL-10 production providing an additional tier of regulation to ensure tightly monitored IL-1β release.
P.05 The fate and plasticity of inflammatory macrophages during peritonitis
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Acute peritonitis leads to transient disappearance of resident peritoneal macrophages and recruitment of large numbers of transcriptionally distinct inflammatory monocyte-derived macrophages. As inflammation resolves, resident macrophages undergo elevated proliferation to reconstitute the cavity but the fate of the inflammatory population remains controversial. Some studies suggest the majority die out while others indicate they convert into resident macrophages. What dictates the balance between death and conversion of inflammatory cells, whether converted cells differ functionally from their bona fide resident counterparts or whether monocytes recruited at the onset of inflammation even retain the ability to differentiate into long-lived resident macrophages remains unclear.

We developed an adoptive transfer system to investigate the long-term fate of inflammatory macrophages. Transfer into a mirroring inflamed environment indicated that inflammatory macrophages present at peak inflammation were able to persist, underwent phenotypic conversion to an F4/80 high MHCII low resident phenotype but remained transcriptionally distinct from bona fide resident macrophages. To ascertain to what extent these differences were cell intrinsic, inflammatory macrophages were adoptively transferred into a cavity depleted of endogenous resident macrophages. Here, inflammatory macrophages adapted a transcriptome much more similar to that of resident macrophages after 8 weeks. Short-term adoptive transfer experiments showed an ability of inflammatory macrophages to adapt a resident phenotype over an 8-day period in the depleted cavity but an inability to do so after transfer into an intact inflamed or naïve cavity.

These experiments indicate that inflammatory macrophages recruited during peak inflammation have the capacity to give rise to mature resident peritoneal macrophages and that this conversion is delayed but not prevented in the presence of incumbent resident cells. Hence, inflammation leads to long-term changes in the origin of the peritoneal macrophage compartment, with a population predominantly seeded during embryogenesis being partially replaced by transcriptionally distinct macrophages differentiated under inflammatory conditions.

P.06 Inflammation in Snakebite Induced Necrosis
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Every year 1.8-2.7 million people living in remote, rural communities fall victim to one of the most neglected tropical diseases; snakebite. Of these, an estimated 137’000 die and 400’000 surviving victims suffer necrosis-induced disfigurements and disabilities that cause severe income and social deprivations.

Whilst a subset of proteins, particularly in viper and spitting cobra venoms (snake venom metalloproteinases (SVMPs), phospholipase A2s, and hyaluronidases) have been implicated in venom-induced necrosis, there is mounting evidence that inflammatory responses can also contribute to pathology. However, understanding of inflammation and snakebite remains in its fledging stages, and the mechanisms that control progression from swelling, to blistering and finally necrosis are poorly understood.
A recent study found that the formation of NETs by neutrophils activated in response to envenomation without necrotic venom toxins in the local tissues prolonging the time they are able to cause degradation and damage. Inflammatory signalling pathways have also been implicated. For instance, SVMP cleavage of inactive membrane bound TNA into its active form promotes the up-regulation and activation of endogenous matrix metalloproteinases, and possibly other uncontrolled pro-inflammatory responses that contribute to local tissue damage.

Using purified venom toxins in in vivo and novel ex vivo assays can help to piece together the puzzle of what these toxins are doing at the bite site, and the involvement of the inflammatory response. An improved understanding of the mechanisms of venom-induced necrosis will help guide anti-toxin immunoglobulin-based approaches to treatment and assess the potential of generic anti-inflammatories to reduce morbidity and improve livelihoods of snakebite victims.

P.07 LC3-associated endocytosis regulates neuroinflammation
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Microglial cells are the resident innate immune cells of the central nervous system (CNS) and are responsible for regulating inflammatory activation in response to both physiological and pathological immune perturbations. The mechanisms that fully control and dictate the nature of an inflammatory response in the CNS are still poorly understood. We have recently identified a novel pathway where components of the canonical autophagy machinery function to conjugate the microtubule-associated protein LC3 to endosomal membranes, a process we have termed LC3-associated endocytosis (LANDO).

We further identified that abrogation of LANDO in microglial cells promotes pro-inflammatory cytokine production in vitro. To characterize LANDO and its role in immune regulation in the CNS, we utilized a well-established model of neuroinflammation induced by β-amyloid deposition. We found that mice lacking LANDO but not canonical autophagy have a robust increase in pro-inflammatory cytokine production in the hippocampus and have increased levels of neurotoxic β-amyloid accumulation. This inflammation and β-amyloid deposition led to reactive microgliosis and hyperphosphorylation of tau, a protein that is vital to neuronal structure and function. As a consequence, LANDO-deficient AD mice have increased neurodegeneration, resulting in impaired neuronal signaling and consequential behavioral and memory deficits. Mechanistically, β-amyloid is internalized by microglial cells through receptor-mediated endocytosis using a variety of surface receptors including Trem2 and CD36. We further identified that LANDO-deficiency not only alters immune activation upon endocytosis of β-amyloid, but results in impaired receptor recycling and extracellular β-amyloid accumulation. Exacerbation of β-amyloid accumulation further contributes to increased neuroinflammation. Together, our data support a protective role for LANDO in microglial cells of the CNS in neurodegenerative pathologies resulting from amyloid deposition. Furthermore, preliminary evidence from other immune cell types and models is suggestive that LANDO is a critical regulator of immune function beyond its role in microglial cells.
P.08 CCR1 regulates branching morphogenesis in the developing mammary gland

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Macrophages are important regulators of branching morphogenesis, a key developmental process in the mammary gland. We have previously demonstrated that the scavenging atypical chemokine receptor, ACKR2 has profound effects on this process by regulating macrophage dynamics during pubertal mammary gland development and embryonic lymphatic vessel development. ACKR2−/− mice display precocious development, associated with increased macrophage recruitment and density of the ductal epithelial network. In embryonic skin, ACKR2 modulates levels of CCL2, a ligand shared with the inflammatory receptor CCR2, which plays a reciprocal role in lymphatic development. However, mammary gland development is unaffected in CCR2−/− mice. Excitingly, we have observed an inverse phenotype, with decreased density of the ductal epithelial network, in the mammary glands of pubertal CCR1−/− mice. CCL7 is a shared ligand between CCR1 and ACKR2, which is elevated in ACKR2−/− glands. Stromal ACKR2 modulates levels of CCL7 to control the movement of macrophages expressing CCR1 to the ductal epithelium. Estrogen is essential for ductal elongation during puberty. Here we have shown that CCR1 is expressed by macrophages in the mammary gland and can be upregulated in response to Estradiol. These data demonstrate that macrophage expression of CCR1 is an important regulator of branching morphogenesis in the mammary gland.

P.09 Helminth-derived inhibitors of the IL-33 pathway

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Allergic asthma is a chronic inflammatory disease of the airways usually characterised by type 2 immune responses in the lungs. Increased mucus production, airway hyperresponsiveness and eosinophilia are the main features of the asthmatic lung, and they are caused by an increased production of IL-5 and IL-13. A strong inducer of these cytokines is the alarmin IL-33 that it is released upon necrotic cell death of epithelial cells. IL-33 release in the lung can be caused by damage caused by inhalation of proteolytic allergens, parasite migration and respiratory viral infection. IL-33 activates several immune cell populations such as mast cells, TH2 lymphocytes and type 2 Innate Lymphoid cells (ILC2s). Parasitic infection is associated with prevention of allergic immune responses, and this prevention appears to be mediated by the release of immunomodulatory excretory/secretory products (ES). The aims of this project are to study ES products from the intestinal murine nematode Heligmosomoides polygyrus (HES), and in particular identifying single proteins that interfere with the IL-33 pathway. In a mouse model of asthma, HES administration has been shown to suppress both IL-33 and ST2 (the IL-33 receptor). We identified two proteins derived from HES which in recombinant form have immunomodulatory activities: HpARI suppresses IL-33 by binding directly to the cytokine while HpBARI blocks ST2.

HpARI binds and blocks IL-33, preventing IL-33-mediated ILC2 activation and production of IL-5 and IL-13, both in vitro and in vivo. HpARI not only suppresses ILC2 activation and reduces eosinophilia in an asthma model using the fungal allergen Alternaria alternata, but also improves lung function and histopathology. In contrast, HpBARI binds to ST2 and blocks the activation of the receptor by IL-33, resulting in suppression of IFN-g, IL-5, IL-6 and IL-13 production in response to IL-33 using in vitro
whole bone marrow cultures. Both molecules will be further characterised to develop possible new
treatment for asthma, while giving insight and a better understanding of the role of the IL-33
pathway in asthma and allergic development.

P.10 Investigating the role of Pez in macrophage wound recruitment

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The use of model organisms is key to develop our understanding of dynamic immunological
processes. The tractable genetics of Drosophila melanogaster, combined with live imaging, provides
an excellent platform to dissect the mechanisms by which immune cells sense and respond to
damage signals released upon wounding. Previous work in the fly embryo has revealed that epithelial
wounding induces the rapid production of hydrogen peroxide (H2O2). This conserved early damage
signal activates the tyrosine kinase Src42a within the embryonic macrophage. Thus, H2O2 triggers an
intracellular phosphorylation cascade – centred around the cell death receptor Draper – which
results in the migration of the macrophage to the wound site. As kinase activation is key to the
response of inflammatory cells in this process, we reasoned that other macrophage specific proteins
may also be phosphorylated in response to H2O2 signalling. Therefore, we conducted a
phosphoproteomics screen in order to uncover novel proteins with altered phosphorylation states
dependent upon activated Src42a. Using this approach, we discovered a wound recruitment defect in
embryos harbouring a mutation in the gene encoding the PTP phosphatase Pez. This was shown to
be cell autonomous via macrophage-specific RNAi expression; and timelapse imaging revealed a lack
of directionality within Pez mutant macrophage in the presence of an epithelial wound. Further, the
use of fluorescent constructs in vivo has shown that Pez localises to sites of active Draper signalling –
such as apoptotic cell uptake. We speculate that Pez may be anchoring active Draper via its N-
terminal FERM domain and/or promoting activated receptor turnover to allow for spatiotemporal
control over downstream signalling. Alongside work in the fly, we are currently extending our study
to Danio rerio. We hope to determine whether the orthologue of Draper – MEGF10 – and ultimately
the orthologue of Pez – PTPN21 – play a conserved role in wound recruitment using tail fin clipping.

P.11 Mutimodal single-cell analysis identifies a novel pro-resolving macrophage subpopulation
following acetaminophen-induced liver injury

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Acetaminophen-induced liver injury (AILI) is the leading cause of acute liver failure (ALF) in western
countries. In severe cases the regenerative capacity of the liver is overwhelmed, resulting in ALF, a
systemic inflammatory response and death. Studies in both humans and animal models have shown
that monocytes/macrophages are key regulators of liver regeneration following AILI. However,
monocytes/macrophages in the context of AILI are heterogeneous and dynamic, exhibiting both pro-
inflammatory and pro-repair phenotypes. Here we used a multimodal approach comprising single-
cell RNA sequencing (scRNA-seq), spectral flow cytometry and immunofluorescence to characterise
myeloid heterogeneity following AILI.
AILI was induced in mice by administration of 300mg/kg acetaminophen. Transcriptome of 31,630 CD45+ cells from heathy and injured livers was analysed by scRNA-seq. Seurat R package was used to cluster myeloid cells. Multiparametric special flow cytometry, immunohistochemistry and multiplex immunofluorescence microscopy were used to characterize the dynamics of parenchymal and non-parenchymal cells following AILI. Phagocytosis was measured using fluorescent dyes: PKH26-PCL and PhagoGreen.

ScRNA-seq revealed four distinct injury specific clusters within the myeloid compartment following AILI, one of which represents a previously unreported subset of monocyte-derived macrophages (CD63+ Ly6CLo MoMFs). Pathway analysis of CD63+ Ly6CLo MoMFs indicated a functional role in necrotic cell clearance, matrix remodelling and tissue regeneration. Using newly identified markers genes, we confirmed expansion of CD63+ Ly6CLo MoMFs during the resolution phase of AILI. Experiments in CCR2-/- and MacBlue-CFP mice indicate CD63+ Ly6CLo MoMFs are bone-marrow derived. These macrophages were spatially located within areas of resolving liver necrosis, adjacent to proliferating hepatocytes. This, in addition to their enhanced phagocytic capacity, suggests these cells drive liver regeneration by promoting clearance of necrotic debris.

Using a multimodal approach, we have dissected the murine hepatic macrophage compartment at unprecedented resolution and identified a novel pro-resolving MoMF subpopulation which expands during liver regeneration following AILI.

P.12 Malaria & that gut feeling: Intestinal inflammation and parasite sequestration during rodent malaria

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Invasive bacterial disease occurs in immunocompromised hosts, including those with malaria. One infection frequently observed in children with Plasmodium falciparum (P.f.) is non-Typhoidal Salmonella (NTS), in which an intestinal infection becomes systemic with serious consequences. While not commonly considered, uncomplicated P. f. infection is associated with intestinal distress – with one study showing 50% of Nigerian children reporting abdominal pain, diarrhoea and vomiting; which clear 3 days after antimalarial treatment. Previously, I have found that rodent malaria (P. yoelii) at 10 days post infection (d.p.i.) can alter the intestinal microbiota and promote colonisation of Salmonella by inducing mild intestinal inflammation in the cecum. However, the mechanism of how malaria induces this localised inflammation is not clear. In this study, we hypothesized that parasitized red cells, which are known to sequester in the tissues during schizogony, can induce inflammation. To test this, we have generated pilot data with GFP-expressing rodent malaria P. chabaudi AS in C57BL/6 mice. At various d.p.i. (4, 7, 11, & 14) during schizogony, the entire intestinal track was excised for analysis by histology, qRT-PCR and microscopy. Here, preliminary results have found that parasite-induced IFNγ and calprotectin (S100a8), was induced before the peak of parasitemia, day 7. Further, histological analysis confirmed increased inflammatory pathology and the presence of the parasite within the intestinal tissue. To understand the consequences of this, we tested systemic organs (liver, spleen, mesLN) for leakage of microbiota by eubacterial 16S qPCR. Here, we found an increase of bacteria in the liver at 4 d.p.i. Together, this preliminary study will help us determine how malaria-induced inflammation promotes dissemination of NTS to systemic sites –
describing both the timing and mechanisms of systemic spread. Therefore, we can begin to ask if ‘invasive’ NTS is; in fact, more invasive or if the host is more permissive?

P.13 Linking Inflammation and Tissue Repair - the Role of the Inflammatory Cells

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Background: Following injury or infection, the initiation of the inflammatory response induces immune cell recruitment and tissue repair. These processes can be dysregulated in diseases, causing damage to the surrounding tissue, thereby disrupting the tissue repair process. Chemotactic mediators such as, formylated peptides, are involved in the recruitment of inflammatory cells. Using fluorescence-activated cell sorted and pure zebrafish (Danio rerio) neutrophils we generated data showing similar morphologies and responsiveness to human neutrophils when exposed to formylated peptides. Zebrafish have the ability to regenerate their tailfin following injury and transgenic strains with labelled immune cells allow us to investigate both the inflammatory response and tissue repair. We hypothesis that the immune cells are key regulators in successful tissue repair and subsequently resolution of inflammation.

Methods: Zebrafish strains with fluorescently labelled neutrophils (tg(mp5:GFPi114)) and macrophages (tg(mpeg1:mCherry)) were used. 3 days post-fertilisation (dpf) the larvae’s tailfins were transected. Simultaneously to the regeneration rate, the migration of the neutrophils and macrophages was measured through serial imaging up to 48 hours post injury (hpi; n>9). Images were analysed using ImageJ with data expressed as mean ± SEM.

Results: The tailfin regenerated ~80% of its pre-cut length by 48hpi. Injured fish show a significant increase in neutrophil numbers 35±3 at 24hpi and 51±5 at 48hpi compared to uninjured fish 27±4 at 24hpi and 31±5 at 48hpi. Macrophage numbers were significantly increased to 45±4 at 24hpi in injured fish compared to uninjured fish 29±4.

Conclusions: Here we demonstrate that neutrophils and macrophages infiltrate the wound early following injury. Tissue regeneration occurs simultaneously suggesting that this influx may be vital for facilitating the tissue repair process. These data also suggest that the morphologies of zebrafish neutrophils are similar to human neutrophils. Currently, we are elucidating the links between inflammation, resolution of inflammation and tissue repair.
Aryl hydrocarbon receptor governs the transcriptional programme of IL-10-producing regulatory B cells

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Regulatory B cells (Bregs) play a critical role in the control of autoimmunity and inflammation. IL-10 production is considered the hallmark for the identification of this population. However, unlike in murine T cells, where it is well established that IL-10 expression is controlled by several transcription factors (TF), the molecular determinants that drive the transcription of IL-10 and suppressive function of Bregs remains unknown. To identify candidate transcription factors (TFs) involved in Breg function, we used IL-10eGFP reporter mice and purified IL-10+ and IL-10−CD19+CD21hiCD24hi B cells and compared their transcriptomes by microarray. We identified the aryl hydrocarbon receptor (AhR) as a key IL-10-associated TF and confirmed AhR binding to the Il10 locus by chromatin immunoprecipitation. Here we demonstrate that AhR controls the differentiation and function of IL-10-producing CD19+CD21hiCD24hi Bregs and prevents their differentiation into B cells that contribute to inflammation. Mice with a B cell-specific deletion of AhR develop an exacerbated arthritis, show an increase in T helper (Th)1 and Th17 cells and a significant reduction in IL-10-producing Bregs and regulatory T cells (Treg) compared to AhR-sufficient mice. A combination of chromatin profiling and transcriptome analyses show that loss of AhR in B cells reduces expression of IL-10 by skewing the differentiation of CD19+CD21hiCD24hi B cells into a pro-inflammatory programme, even under Breg-inducing conditions. Thus, AhR acts as a transcriptional regulator of Breg differentiation by implementing a molecular programme that controls B cell IL-10 production and represses pro-inflammatory cytokine production.

Imaging extracellular vesicles arising from apoptotic tumour cells

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Apoptosis is a form of programmed cell death with diverse effects on inflammatory mechanisms including those of significance in the tumour microenvironment. Emerging data show that, besides its role in tumour suppression, apoptosis can also promote oncogenic progression. The network of regeneration and tissue repair mechanisms driven by cell-death in tumours has been termed the “onco-regenerative niche” and we hypothesize that amongst other elements, extracellular vesicles (EV) are key components of this network. EV are membrane structures secreted by cells, containing multiple types of bioactive material, including markers of the cells they originate from. EV show numerous applications in healthcare and diagnostics, and there is an increasing research interest in their biological functions. However, mainly because of their small size and heterogeneity, there are challenges associated with their analysis, and although EV show increasing popularity in the clinical diagnostic practice, the guidelines for analytic procedures have not been established to date [4]. Here, we present analytical techniques which can be applied to the nano-scale for imaging EV in pre-
clinical research and with the potential for application to patient samples. We investigate EV released in vitro by apoptotic Burkitt lymphoma cells (apo-EV) in relation to their tumour-regulating effects. Basic physical-chemical properties of apo-EV such as structure, size distribution, surface charge and membrane fluidity are discussed, followed by a comparison of methods for the analysis of the surface markers (immunophenotyping).

It is concluded that for the characterization of the heterogenous EV populations, comparison of results from multiple techniques is often required, and also, understanding the strengths and limitations of each method is essential for choosing the appropriate combination of analytical tools. Finally, we consider that the monitoring of such EV and their cargoes is a promising tool to help to improve cancer diagnosis, prognosis and evaluation of therapeutic efficacy.

P.16 Granzymes protect against Salmonella Typhimurium oral infection

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Granzymes are cytotoxic proteases that are an integral part of the arsenal used by cytotoxic immune cells to kill infected/damaged cells. However, previous studies indicate redundancy in the Granzyme family, as mice deficient in either Granzyme A or B are only mildly susceptible to virus or intracellular bacterial infections, when challenged intravenously. Through quantitative mass spectrometric analyses, we found that Granzymes A and B are very highly expressed in intestinal intraepithelial lymphocytes (IEL). Indeed, IEL are the main immune cells expressing Granzymes in healthy non-infected mice. IEL play a crucial role in regulating intestinal homeostasis. They preserve the intestinal barrier integrity and patrol the epithelial layer to sense and protect against intestinal infection. However, the exact mechanisms by which IEL protect against foodborne pathogens is still unclear. To investigate whether Granzymes are important for the anti-bacterial functions of IEL, we are using the well-described Granzyme A and/or B deficient mice (Gzma-/-, Gzmb-/- and Gzma-/-Gzmb-/-). When orally challenged with Salmonella Typhimurium, Gzma-/-Gzmb-/- mice are unable to clear the bacteria, and also show signs of heightened inflammatory responses within days of infection. Moreover, Salmonella infections in either Gzma-/- or Gzmb-/- mice indicate that Granzyme A and Granzyme B play different roles in early and late salmonella pathogenesis. The exact role of IEL in this phenotype is still being investigated but our data highlight an important and previously overlooked role of granzymes in the protection against oral infection.
Session 1: Tissue damage and loss of homeostasis

P.17 cGAS detection of micronuclei links immune surveillance to autoinflammation

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Aicardi-Goutières syndrome (AGS) is a rare genetic disease which causes devastating brain inflammation in children and is associated with a Type 1 Interferon response. It can be viewed clinically as a genetic mimic of congenital viral infection. Mutations in ribonuclease H2 (RNase H2) genes, coding for an enzyme that is vital for genome stability, are the most common cause of AGS. We, and others, have previously shown that immune activation in RNase H2 deficiency is associated with triggering of the cytosolic DNA sensor cGAS and its adaptor protein STING. This observation raised the critical question of how cGAS is activated in RNase H2 deficiency given that DNA is strictly compartmentalised within the nucleus.

Here, we describe how cGAS localises to structures known as micronuclei within RNase H2 deficient cells. Micronuclei are formed during mis-segregation of DNA during mitosis. This DNA is then surrounded by its own nuclear membrane forming a structure that is discrete from the primary nucleus. Micronuclei are increased in the presence of genome instability and are often found in human cancer cells. We find that cGAS localisation to micronuclei is not restricted to RNase H2 deficiency, also occurring following exogenous irradiation-induced DNA damage and in micronuclei arising spontaneously in human cancer cells. Using live-cell imaging we show that breakdown of the micronuclear envelope results in rapid accumulation of cGAS, providing an explanation of how cytosolic cGAS gains access to this immunostimulatory DNA. Using live-cell laser microdissection and single cell transcriptomics we find that interferon-stimulated gene expression is preferentially induced in cells with micronuclei. We thus describe a novel cell-intrinsic immune surveillance mechanism that could detect a range of neoplasia inducing processes, but where aberrant activation triggers autoinflammatory disease [1].


P.18 Central nervous system regeneration is driven by microglia death and repopulation

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The prime example of effective regeneration in the central nervous system is that of remyelination, whereby re-ensheathment of nerve fibers with the insulating sheath termed myelin restores electrical impulse conduction and trophic/ metabolic support. Remyelination fails in a multitude of neurological disorders, which is considered to contribute to nerve damage/ loss, correlating to clinical decline. The lack of approved therapies promoting remyelination highlights the need to
elucidate the underpinning mechanisms. Our previous work showed that efficient remyelination requires dynamic regulation of microglia activation, with a transition from a pro-inflammatory (iNOS+ TNF-alpha+ CD16/32+) to a pro-regenerative phenotype (Arg-1+ CD206+ IGF-1+). The chronic pro-inflammatory microglia activation commonly observed in neurological disorders suggests an impairment in this transition. However, the cellular and molecular mechanisms regulating the activation of microglia and resolution of inflammation are unknown. Using a combination of ex vivo and in vivo modelling of myelin damage, live imaging of microglia dynamics, and correlation to human CNS pathology, we unveiled that remyelination is driven by coordination of pro-inflammatory microglia death and repopulation to a regenerative state. Death was found to occur by controlled necrosis (necroptosis), which when blocked, lead to the persistence of pro-inflammatory microglia and impairment of remyelination. Lineage tracing revealed that repopulation was mediated by two distinct sources -- residual microglia that did not die and a central nervous system-resident stem cell -- and required type I interferon signalling. Additionally, microglia death and repopulation were associated with remyelination efficiency in human neurological disease. In summary, we propose that failed remyelination may represent an impairment in microglia death and/or repopulation, and that targeting these processes may represent a novel strategy to dampen chronic neuroinflammation and promote central nervous system regeneration.

P.19 Microbiotas from Humans with Inflammatory Bowel Disease Alter the Balance of Gut Th17 and RORyt+ Regulatory T Cells and Exacerbate Colitis in Mice

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A general consensus exists that inflammatory bowel disease (IBD) is associated with compositional and metabolic changes in the intestinal microbiota. However, a barrier to considering microbiota manipulation as a treatment for IBD is a lack of evidence showing a causative influence of the IBD-associated microbiota on immune function and inflammation. To address this, we investigated the impact of fecal microbiota from patients with IBD on gut immune responses in gnotobiotic mice. Germ-free mice were colonized with fecal microbiotas from 15 human donors with IBD and 15 healthy donors. We used flow cytometry to identify homeostatic gut immune phenotypes induced by each donor and defined the effect of each donor microbiota on immune function and inflammation. To address this, we investigated the impact of fecal microbiota from patients with IBD on gut immune responses in gnotobiotic mice. Germ-free mice were colonized with fecal microbiotas from 15 human donors with IBD and 15 healthy donors. We used flow cytometry to identify homeostatic gut immune phenotypes induced by each donor and defined the effect of each donor microbiota on the susceptibility of gnotobiotic mice to intestinal inflammation in a colitis model. In unchallenged mice, microbiotas from donors with IBD promoted a different immunological tone in gut tissue than healthy donor microbiotas. IBD microbiotas induced a higher proportion of Th17 cells than microbiotas from healthy donors. We also observed that healthy donor microbiotas potently induced a subset of FoxP3+Treg co-expressing RORyt. Compared to healthy donors, microbiotas from donors with IBD induced a lower proportion of RORyt+Treg. Using the Rag-deficient T-cell transfer colitis model, we observed that mice colonized with microbiotas from donors with IBD develop more severe colitis than mice colonized with microbiotas from healthy donors. The severity of colitis transferred by a microbiota was correlated with the capacity of that microbiota to induce Th17 cells and inversely correlated with induction of RORyt+Treg cells in wildtype animals. In conclusion, we find that fecal microbiotas from individuals...
Session 2: Immune modulation

P.20 Cathelicidin is a “fire alarm”, generating protective NLRP3-dependent airway epithelial cell inflammatory responses during infection with Pseudomonas aeruginosa

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Pulmonary infections are a major global cause of morbidity, exacerbated by an increasing threat from antibiotic-resistant pathogens. In this context, therapeutic interventions aimed at protectively modulating host responses, to enhance defence against infection, take on ever-greater significance. Pseudomonas aeruginosa is an important multidrug-resistant, opportunistic respiratory pathogen, the clearance of which can be enhanced in vivo by the innate immune modulatory properties of antimicrobial host defence peptides from the cathelicidin family, including human LL-37. Initially described primarily as bactericidal agents, cathelicidins are now recognised as multifunctional antimicrobial immunomodulators, modifying host responses to pathogens, but the key mechanisms involved in these protective functions are not yet defined. We demonstrate that P. aeruginosa infection of airway epithelial cells promotes extensive infected cell internalisation of LL-37, in a manner that is dependent upon epithelial cell interaction with live bacteria, but does not require bacterial Type 3 Secretion System (T3SS). Internalised LL-37 acts as a second signal to induce inflammasome activation in airway epithelial cells, which, in contrast to myeloid cells, are relatively unresponsive to P. aeruginosa. We demonstrate that this is mechanistically dependent upon cathepsin B release, and NLRP3-dependent activation of caspase 1. These result in LL-37-mediated release of IL-1β and IL-18 in a manner that is synergistic with P. aeruginosa infection, and can induce caspase 1-dependent death of infected epithelial cells, and promote neutrophil chemotaxis. We propose that cathelicidin can therefore act as a second signal, required by P. aeruginosa infected epithelial cells to promote an inflammasome-mediated altruistic cell death of infection-compromised epithelial cells and act as a “fire alarm” to enhance rapid escalation of protective inflammatory responses to an uncontrolled infection. Understanding this novel modulatory role for cathelicidins, has the potential to inform development of novel therapeutic strategies to antibiotic-resistant pathogens, harnessing innate immunity as a complementation or alternative to current interventions.
**P.21 The β2 integrin, LFA-1, is a novel regulator of IL-17-producing γδ T cells**

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γδ T cells are innate-like cells that reside predominantly at mucosal sites and have essential roles in immune surveillance. They produce pro-inflammatory cytokines and kill target cells, protecting the host from infection and cancer. Despite recent advances in the development of γδ T cell therapy for cancer treatment, our understanding of the basic biology of these cells remains poor. Here, we have identified the adhesion molecule, LFA-1 (integrin αL/β2; CD11a/CD18), as a novel regulator of γδ T cells. In the absence of LFA-1 (in CD11a or CD18 KO mice), γδ T cells are significantly expanded. Extensive analysis of mucosal and lymphoid tissues revealed that this is not redistribution of γδ T cells as a result of a homing defect, but a tissue-specific expansion: 50-fold in lungs and spleen; 8-fold in uterus; whereas numbers in the skin, gut and oral mucosa were normal. In mice, specific γδ T cell subsets are identified by Vγ chain expression, surface markers and cytokine profile. Our findings show a specific increase in IL-17-producing Vγ6+Vδ1+ γδ T cells in CD18 KO mice. These cells localise to the lungs and uterus, which fits with our original findings of γδ T cell expansion at these sites. Finally, we investigated the mechanism of expansion. Although we found no evidence of enhanced development or proliferation, we found that CD18 KO Vγ6+ γδ T cells expressed reduced levels of the pro-apoptotic molecule, Fas. These findings suggest that the accumulation of Vγ6+ γδ T cells in CD18 KO mice is due to increased survival. Together, our data reveal a novel role for LFA-1 in regulating γδ T cell subset numbers in the periphery. Further work is now required to determine the functional outcomes of Vγ6+ cell expansion, with the aim to help inform the use of γδ T cells in cancer therapeutics.

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**P.22 Omental fat-associated lymphoid clusters constrain peritonitis through neutrophil recruitment and targeted NET release**

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The omentum is a visceral adipose tissue that provides a first layer of immunological protection within the abdomen by supporting IgM secreting innate-like B cells within Fat-associated lymphoid clusters (FALCs). Single-cell RNA sequencing of FALC stromal cells coupled with subsequent spatial analysis, revealed that the surface of omFALCs are covered with a subset of mesothelial derived stromal cells (which we name as FALC Cap cells) specialised in the early recruitment of neutrophils and monocytes. In vivo during murine peritonitis, neutrophils are rapidly recruited to the surface of omFALCs where they release extensive neutrophil extra-cellular traps (NETs) in a CXCL11 and PAD4 dependent manner. This process permitted the omentum to efficiently trap bacteria. In humans with acute appendicitis, omentum-neutrophil-NET interactions resulted in specific and localized bacterial capture. Therefore, FALC-targeted NET release is an important immunological mechanism ensuring the barrier function of the omentum to peritoneal infection and subsequent abdominal sepsis.
In 1907 Dr. Alois Alzheimer published his research on the histological features of a patient who had dementia which initially presented as severe memory deficits and progressed into other cognitive faculties. Dr. Alzheimer described three main histological features of what is now termed Alzheimer’s disease; these included extracellular and intracellular protein aggregates as well as clusters of non-neuronal cells. For the better part of the following century, research focused on the protein aggregate features of the disease. Recently, from a consensus of neuroimaging, histological, genomic and preclinical research, it is now clear that the previously overlooked feature of non-neuronal cell clusters are neuroinflammatory microglia and astrocytes, and this inflammatory process is central to the pathophysiology of Alzheimer’s disease. However, it is also clear that not all microglial and astrocyte functions are deleterious and so establishing the key physiological switches which dictate the pathological phenotype of these neuroimmune cells is critical. Cell based experiments and genetic mouse models identified the cytosolic pattern recognition receptor NLRP3 as the sensing molecule of protein aggregates present in Alzheimer’s disease and following activation, the NLRP3 receptor mediates the release of the highly inflammatory cytokine interleukin-1β (IL-1β). This process has been identified as an essential deleterious switch in the neuroimmune pathological response. Therefore, we investigated if any existing non-steroidal anti-inflammatory drugs (NSAIDs) had any inhibitory effects on NLRP3 activation in bone marrow derived (BMDM) macrophages in vitro. From screening numerous compounds we found that a group of structurally similar NSAIDs did inhibit NLRP3 activation; including flufenamic acid, mefenamic acid and diclofenac. We then translated the lead compound mefenamic acid into two animal models of Alzheimer’s disease. Mefenamic acid treatment abated memory deficits in an amyloid oligomer intraventricular injection rat model of amyloid toxicity and the 3xTg mouse model of familial AD. Using immunohistochemistry we also saw significant reductions in amoeboid – IL-1β expressing inflammatory microglia. To investigate the human relevance of this discovery, we then performed epidemiological analyses on the Alzheimer’s Disease Neuroimaging Initiative longitudinal dataset which contains medication and cognitive function information on 1619 individuals. Using innovated statistical methods we identified that the majority of NSAIDs had no effect on cognitive decline. However, diclofenac, the only NLRP3 inhibiting NSAID used in the cohort, significantly slowed cognitive decline. Collectively, this research has identified a subgroup of NSAIDs as potential therapeutics in Alzheimer’s disease, potentially through the inhibition of NLRP3 and subsequent IL-1β release.
Session 3: Immune modulation

P.24 Pro-inflammatory embryonic macrophages are essential components of the hematopoietic stem cell generative microenvironment

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The hematopoietic stem cells (HSC) responsible for the life-long maintenance of the adult vertebrate blood system are generated from specialized endothelial cells in the embryonic aorta. Whereas some studies implicate inflammatory factors in the development of HSCs, the cells responsible for this inflammatory microenvironment have not been characterized. Macrophages play pro- and anti-inflammatory roles during adult life, and during embryonic development function mainly in tissue remodeling. However, it is not known if macrophages, which are made prior to HSC generation, affect the development of the hematopoietic system. We used CyTOF to show the distinct phenotypes of aorta-gonad-mesonephros-associated macrophages (AGM-aM) and identified two myeloid cell types differing in mannose receptor expression. Fully differentiated AGM-aM are mannose receptor positive and, in contrast to what is typically observed in the adult, express a pro-inflammatory signature. We found that AGM-aM emigrate from the yolk sac through the CX3CR1/CX3CL1 axis, localize to the embryonic aorta and interact with nascent intra-aortic hematopoietic cells (IAHC). Importantly, upon ablation of AGM-aM, no long-term repopulating HSCs are detected, thus demonstrating that a unique subset of yolk sac-derived inflammatory macrophages play an essential role in regulating the embryonic development of adult HSCs.

P.25 Reintroduction of pro-resolving factors issued from efferocytosis terminate ongoing experimental IBD affecting both inflammation and mucosal lesion healing

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Inflammation is a natural body defense reaction in response against injuries. Due to some environmental and/or genetic factors, the process of inflammation resolution could be dysregulated and initiate the development of inflammatory chronic diseases, as in chronic inflammatory bowel diseases (IBD). Most of IBD patients are refractory or present a loss of response to treatment resulting in severe and complicated disease. New approaches are thus necessary. We have developed a new approach based on the reintroduction of pro-resolving factors, naturally produced during efferocytosis. This treatment favored the complete resolution of experimental arthritis for instance. Here we addressed whether this treatment call SuperMApo®, essentially composed of cytokines, chemokines and lipids could stop ongoing IBD affecting both inflammation and mucosal lesions. We used two preclinical experimental models, one based on the transfer of CD4+CD45RBhighCD25– naïve T cells into immunodeficient C57Bl/6 RAG2−/− mice and a second one based on the oral administration of dextran sodium sulfate (DSS) 3% in the drinking water of C57Bl/6 mice. Through the association of mouse clinical score and live endoscopic evaluation, we demonstrated that SuperMApo® injection in IBD mice induced a rapid and significant decrease in both the clinical and endoscopic scores (p <0.05) in both models. This was associated with a higher
proliferation of mucosal cells within the crypts and reprogramming of fibroblasts to pro-healing fibroblasts. We identified the factors within SuperMApo® associated with mucosal wound healing. In addition, we observed pro-Treg macrophages and dendritic cells within the spleen and, in the colon, a decrease of Th1 T cells and dendritic cell expressing PD-L1. Interestingly, higher healing properties of fibroblasts were observed ex vivo using biopsies from IBD patient and Human SuperMApo®. Altogether, our preliminary data suggest that SuperMApo® should be efficient to resolve ongoing inflammation and heal mucosal lesions in IBD refractory patients.

P.26 Mitochondria as inflammatory killers
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Diverse anti-cancer therapies kill cancer cells via mitochondrial apoptosis. Nevertheless, apoptotic resistance - either intrinsic or acquired - remains a key barrier to effective cancer therapy. Addressing this, we have recently found that the way in which a cancer cell dies massively impacts on therapeutic efficacy.

During mitochondrial apoptosis, mitochondrial outer membrane permeabilisation (MOMP) occurs; this allows the release of mitochondrial proteins that activate caspase proteases and apoptosis. However, irrespective of caspase activity, cells still die following MOMP, defining it as a point-of-no-return. Mitochondrial apoptosis is considered immunologically silent. In contrast, we find that under caspase inhibited conditions, cell death following MOMP is pro-inflammatory. Underpinning this, mitochondria drive a variety of pro-inflammatory signalling pathways, including NF-kB and cGAS-STING. Importantly, we find that killing cancer cells under caspase inhibited conditions leads to induction of anti-tumour immunity and tumour eradication.

Collectively, our data demonstrate that mitochondria are inherently pro-inflammatory during apoptosis and that caspases serve to silent this effect. Secondly, they imply that inhibiting apoptotic caspase function may be an effective anti-cancer therapeutic approach.

P.27 The secreted protein Del-1 promotes resolution of inflammation
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Acute inflammation is initiated by neutrophil and monocyte recruitment to the inflamed site, followed by release of pro-inflammatory mediators. After their acute proinflammatory actions, recruited neutrophils undergo apoptosis and are removed by macrophages through efferocytosis. Macrophage engulfment of the apoptotic material leads to the induction of a downstream signaling in which efferocytic macrophages acquire a resolving phenotype by releasing TGFβ1 and by further upregulation of phagocytic receptors and bridging molecules. Resolving macrophages are central to resolution of inflammation, which is essential for tissue homeostasis. We have previously described
Developmental endothelial locus-1 (Del-1) as an endogenous negative regulator of leukocyte adhesion.

To investigate the role of Del-1 in the modulation of inflammation resolution, we employed two different animal disease models, used genetic and pharmacological approaches and also analyzed relevant human samples.

Here, we show that Del-1 also operates as an endogenous pro-resolving mediator. In human and murine oral inflammatory disease, periodontitis, resolution of inflammation correlated with Del-1 upregulation, whereas resolution of experimental periodontitis failed in Del-1 deficiency. This concept was generalized and mechanistically substantiated in acute monosodium urate crystal-induced inflammation, where the pro-resolution function of Del-1 was attributed to effective efferocytosis. Consistent to its ability to resolve inflammation, Del-1–mediated efferocytosis induced macrophage reprograming to a resolving phenotype in a manner dependent on nuclear receptor liver X receptor. Importantly, by generating different transgenic mice with cell-specific overexpression of Del-1, we ascribed the anti-leukocyte recruitment action to endothelial cell-derived Del-1 and the efferocytic/pro-resolving action to macrophage-derived Del-1.

Therefore, the homeostatic function of Del-1 is not restricted to regulating initiation of inflammation but also encompasses its resolution and this versatility is facilitated by the compartmentalized expression of Del-1 that enables it to perform distinct functions in an appropriate context that can be harnessed therapeutically.
**Poster Abstracts - Thursday 24 April & Friday 25 April**

**P.28 Multiomic definition of generalizable inflammation endotypes in human acute pancreatitis**

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Acute pancreatitis (AP) is sudden onset pancreas inflammation that causes multiple organ dysfunction syndrome (MODS) and death in certain individuals, yet minimal systemic inflammation in others. Here, we show that this observed diversity in systemic response and outcome is accompanied by diversity in molecular subtypes that can be identified using computational analysis of clinical and multiomic data. We integrated co-incident whole blood transcriptomic, plasma proteomic, and serum metabolomic data at serial time points from a cohort of patients presenting with AP and systematically evaluated four different metrics for patient similarity, using unbiased mathematical, biological and clinical measures of internal and external validity. Our results identify four distinct and stable AP endotypes that are characterized by pathway and biomarker combination stereotypes into hypermetabolic, hepatopancreaticobiliary, catabolic and innate immune endotypes. The catabolic endotype in AP strikingly recapitulates a disease endotype previously reported in acute respiratory distress syndrome, a recognized complication of AP. Our findings demonstrate that clinically-relevant and generalizable inflammation endotypes exist during systemic inflammation in AP.

**P.29 TREM2 controls CNS myeloid cell accumulation and transcriptome reprogramming to promote injury resolution and functional recovery after stroke**

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Understanding how the brain remolds and adapts to damage caused by stroke is essential to develop alternative therapeutic approaches. Growing evidence suggests that specific subsets and phenotypes of microglia and CNS macrophages can support tissue repair and recovery of function. Endogenous mechanisms controlling these phenotypes will be important to determine as a basis for developing interventions. We have investigated the involvement of TREM2, a cell surface immunomodulatory receptor expressed on myeloid cells, in injury resolution, brain repair and functional recovery using a model of experimental stroke in mice.

Experimental stroke was induced in wild-type or TREM2-/- mice using the permanent middle cerebral artery occlusion method. At various timepoints during the acute, subacute and chronic phases, a range of histological, molecular/transcriptomic and functional analyses were performed. Chimaeric mice were generated with TREM2 expression restricted to microglia to enable the contribution of TREM2 in different myeloid compartments to be determined.

TREM2 expression was induced in the subacute phase after stroke. Reactive microglia and macrophages accumulated around the peri-infarct border however this was suppressed in TREM2-/- mice and significantly fewer microglia and monocyte-derived macrophages (CCR2+ and CCR2-) were
present in the injured hemisphere of TREM2-/- mice. Subacute injury resolution and functional recovery were also impaired in TREM2-/- mice and chimaeric studies showed this was dependent on microglial-derived TREM2 expression. The induction of transcriptional networks controlling myeloid cell activation, angiogenesis and matrix remodelling were blunted in TREM2-/- mice.

Our findings emphasise the importance of appropriate myeloid cell reactivity to promote injury-induced brain remodelling and subacute functional recovery and demonstrate TREM2 as a key endogenous regulator. Developing approaches to harness/augment TREM2-driven pathways and more generally pro-regenerative CNS myeloid cell activity may offer new opportunities to treat stroke patients.

P.30 Manipulating brain macrophages via CSF1R to enhance brain repair and functional recovery after stroke
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Introduction: Stroke is a leading cause of death and disability worldwide. Current reperfusion treatments for acute stroke are available to <10% of patients. A major unmet clinical need is to develop alternative and/or adjunct approaches that target mechanisms distinct from reperfusion therapy. A key strategy is to improve the capacity of the damaged brain to adapt to the primary injury and to stimulate repair and functional recovery. Increasing evidence indicates that macrophages can drive tissue repair processes. An important challenge is to identify tools that can harness the reparative roles of brain macrophages in CNS injury. Colony stimulating factor-1 (CSF1) is a key regulator of myeloid cell survival, trafficking, differentiation and phenotype. We explored a modified version of CSF1 as a candidate pro-regenerative treatment after experimental stroke.

Methods: C57BL/6J adult male mice underwent experimental stroke induced by middle cerebral artery occlusion (MCAO) or sham surgery. Mice received vehicle or CSF1-Fc treatment initiated after MCAO onset. A range of cellular, tissue and functional assays were assessed.

Results: CSF1-Fc treatment (1) improved functional recovery 3 days after MCAO, (2) altered peri-infarct microglial/monocyte-derived macrophage numbers and morphology, (3) promoted pro-regenerative phenotypic alterations in microglia in vitro, (4) penetrated the blood-brain-barrier after MCAO, and (5) did not induce markers of hepatic or renal toxicity.

Conclusions: Our data indicate efficacy, target bioactivity and biosafety of CSF1-Fc in a preclinical stroke model, and support CSF1-Fc as a candidate pro-regenerative treatment after stroke via effects on systemic and/or brain macrophage populations. More broadly, our results reinforce that augmenting macrophage accumulation/activity can have beneficial effects in the injured brain. Future work will seek to define the phenotypic changes that may underpin the recovery-enhancing effects of CSF1-Fc treatment.
**P.31 Reciprocal functions of prostaglandin on intestinal homeostasis and inflammation**

**Chengcan Yao**

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Prostaglandin E2 (PGE2) plays critical roles in various physiological and pathophysiological processes by interacting with its receptors EP1-4. Non-steroidal anti-inflammatory drugs, by targeting cyclooxygenases and inhibiting prostaglandin production, are widely used to treat both acute (e.g. pain/fever) and chronic inflammation (e.g. arthritis). But these drugs can have also severe adverse effects such as gastrointestinal bleeding. We have observed that blockade of PGE2 synthesis induces gut barrier disruption and bacterial translocation, resulting in systemic inflammation and exacerbated acute intestinal injury. This could be prevented by activation of EP4, indicating that PGE2-EP4 signaling controls gut barrier injury, bacterial translocation and systemic inflammation.

Mechanistically, PGE2-EP4 signaling maintains intestinal type 3 innate lymphoid cell (ILC3) homeostasis and stimulates IL-22 production. We thus demonstrated the fundamentally physiology role of PGE2 in maintaining intestinal homeostasis and offered an interpretation for the well-known gastrointestinal side effects of NSAIDs.

On the other side, however, genome-wide association studies have revealed that polymorphisms in the PTGER4 gene (encoding EP4) loci are associated with EP4 overexpression and more severe disease phenotype in patients with Crohn’s disease. By analyzing T cell responses in the intestine, we have found that PGE2 controls intestinal Treg responses through gut microbiota-dependent modulation of mononuclear phagocytes, leading to facilitating intestinal inflammation. The findings in experimental mouse models are corroborated by gene expression data from human intestinal biopsy samples taken from patients with Crohn’s disease. This work thus suggests that PGE2-EP4 signalling may play a critical role in the pathogenesis of chronic intestinal inflammation, and that targeting the PGE2-EP4 pathway may be a promising therapeutic strategy for control of some intestinal inflammatory condition.

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**P.32 Macrophages in endometriosis exhibit phenotypic heterogeneity and have potential as therapeutic targets**

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Endometriosis is a chronic inflammatory disorder associated with pelvic pain and infertility that affects ~176 million reproductive age women worldwide. It is defined by the presence of endometrial-like tissue outside the uterus (lesions). Current management is unsatisfactory and there is an unmet need for new treatments. Macrophages play a key role in the growth, vascularization and innervation of endometriosis lesions, however our knowledge of macrophage origins, phenotype and heterogeneity in endometriosis is limited. We aimed to define the origin of lesion-resident macrophages and assess phenotypic heterogeneity using our unique mouse model. To assess infiltration of tissue-resident large peritoneal macrophages (LpM) into lesions, we adoptively
transferred LpM (isolated from Csf1r-EGFP mice) into the peritoneal cavity of endometriosis mice. GFP+ cells were identified by immunohistochemistry in lesions after 2 weeks (n=4). To assess infiltration of Ly6Chi monocytes into lesions we performed Ly6C, F4/80 dual immunofluorescence. We identified both Ly6C+ monocytes and F4/80+ mature macrophages in lesions, which was also validated using flow cytometry (n=6). Next, we induced endometriosis in WT mice using donor endometrial tissue from Csf1r-EGFP mice; we identified that 16% (SEM± 4%) of lesion macrophages were GFP+ endometrial-derived macrophages (n=6). Using single-cell RNA-seq, we performed unbiased transcriptional profiling of CD45+ cells in lesions (6006 cells), donor endometrial tissue (1306 cells) and peritoneal lavage cells from sham (5645 cells) and endometriosis mice (6720 cells). Clustering analysis identified 4 lesion-resident macrophage populations. Comparison of lesion-resident macrophage populations with macrophage clusters from endometrial tissue and the peritoneum confirmed that lesions contained macrophages from different origins. In summary, we demonstrate, for the first time, that macrophages in endometriosis lesions are heterogeneous in both ontogeny and transcriptional profile. We believe that this observed heterogeneity could be translated into clinical applications, such as targeted therapy for endometriosis-associated pain.

P.33 NF-κB p50 phosphorylation and the regulation of Toll-like receptor induced in human macrophages

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NF-κB is a family of transcription factors formed by 5 members: RelA, RelB, c-Rel, p52 and p50. They play a critical role in the control of inflammation, and the phosphorylation of NF-κB is a key mechanism for the regulation of target gene transcription through the modulation of interaction with co-activators, chromatin modifying enzymes, other transcription factors and altering protein stability. However much of what we know comes from the study of the RelA subunit and relatively little is known about the phosphorylation of the NF-κB p50 subunit and how it contributes to the regulation of pro-inflammatory gene expression. The p50 subunit of NF-κB is a critical factor in human inflammatory disease and functional polymorphisms in p50 are significant risk factors for the development of ulcerative colitis, acute respiratory distress syndrome, systemic lupus erythematosus, COPD, autoimmune syndromes, as well as a number of cancers.

In this study we investigated the role of p50 phosphorylation in the regulation of innate immune transcriptional responses. We have employed CRISPR/Cas9 gene editing approaches to generate for the first time THP1 human monocyte cell lines that either lack the p50 gene (NFKB1-/-) or in which the specific p50 phospho-acceptor sites S73, S74, S80, S328, S337, Y409 have been mutated. THP1-derived macrophages expressing phosphosite mutants of p50 were stimulated with LPS and analysed by RNA-seq to identify the transcriptional signatures associated with phosphorylation at each site.

This revealed a unique gene signature associated with each phosphorylation, demonstrating distinct functional roles for individual p50 phosphorylation sites. Phosphorylation sites in close proximity to each other showed a significant overlap in the genes regulated indicating that they may share a common regulatory mechanism. These data advance our understanding of gene specific mechanisms of transcriptional control by providing a molecular basis for gene selective regulation of transcription by p50 phosphorylation.
Extracellular matrix mediated control of the immune response

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Chemokines are central to leukocyte recruitment and associated inflammatory based pathologies, e.g. atherosclerosis and autoimmune disease. Chemokine function is dependent on their ability to bind to cell surface glycosaminoglycan (GAG) sugar chains. GAGs are extracellular matrix components that support chemokine localisation and help to form the glycocalyx on the apical surface of the endothelium, a physical barrier to leukocyte adhesion and migration. How these factors collaborate to enable leukocyte migration is yet to be understood, precluding therapeutic targeting of chemokines during inflammatory disease.

We hypothesise that:

1. Chemokine oligomerisation enables interactions with GAGs and re-modelling of the glycocalyx to enable leukocyte recruitment.
2. Modification of glycosaminoglycan chemistry confers specificity onto the “apparently” redundant chemokine system.

We utilized biophysical techniques and endothelial binding to examine the interaction of chemokines, and mutants, with different isolated GAGs and the cell surface. Biological relevance has been investigated using the air pouch model of inflammation. Our results demonstrate that chemokines bind to GAGs with a range of affinities, and specific GAG decoration enables presentation of certain chemokines, possibly conferring tissue specificity. Chemokine oligomerization also plays a key role in their presentation on the endothelial cell surface to circulating leukocytes. Our data also suggests that chemokines can re-structure key glycocalyx components (HS chains) on a biosensor. This process may provide a novel mechanism for how chemokines enable recruitment of leukocytes to tissues from the circulation and confer specificity to an apparently redundant system.

We conclude that oligomerization enables binding and modification of GAG chains, enhancing cell surface retention and facilitating chemokine mediated GAG re-organization. This mediates chemokine cell-surface localization under flow and thus leukocyte recruitment in vivo; potentially suggesting a novel function of chemokines in physical re-organization of the glycocalyx. This represents just one facet of the emerging role of proteoglycan sugar chains in regulating inflammation.

RNA sequencing of embryonic macrophages in the haematopoietic stem cell generative microenvironment

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Haematopoietic stem cells (HSCs) are responsible for the life-long maintenance and regeneration of the adult vertebrate blood system. Haematopoietic development in the vertebrate embryo occurs in several waves, with each sequential wave producing cohorts of cells that are increased in complexity of blood lineage potential. HSCs arise in the AGM (Aorta Gonad Mesonephros) region {Medvinsky, 1996} and other major arterial vessels {de Bruijn, 2000} through a transdifferentiation process called
endothelial-to-haematopoietic transition (EHT) (Dzierzak, 2018; Jaffredo, 1998). Embryonic macrophages from the first two waves function in different developmental processes. However, it is not known if macrophages, which are made prior to HSC generation, affect the development of the haematopoietic system.

In order to study their innate cellular composition during the highly dynamic and crucial induction of the HSC niche, RNA sequencing was carried out upon the HSC-generative microenvironment. AGM, Yolk sac (YS) and Hindbrain (HBA) GFP+CD206+ and GFP+CD206- populations from MacGreen (Csf1rGFP) transgenic mouse E10.5 embryos were FACS sorted and sequenced as per the Smart-seq2 protocol. Datasets from these MacGreen populations were compared (3 biological replicates per population). Unsupervised principal component analysis (PCA) separated the CD206+ and CD206- cells into two distinct clusters for each of the embryo sites. Further PCA clustered populations of macrophages from different sites in the embryo suggesting the migration and maturation of particular populations within the embryo. Differential expression analysis between populations (n=10) identified ≈242 genes (out of ≈20,000) significantly altered in CD206+ macrophages compared to CD206- cells. Gene ontology studies for enriched genes highlighted inflammatory gene signatures and distinct receptor signalling activity. We are currently working on validating these findings in vitro.

In summary, this work sheds light on the understanding of the role of a novel CD206+ macrophage during HSC induction.

P.36 Optimisation of a Novel Flow Cytometry Assay for the Efficient Detention and Quantification of Neutrophil Extracellular Traps (NETs)

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Neutrophil extracellular traps (NETs), referred to as NETosis. NETs represent a ROS-dependent cell death process during which neutrophils release nuclear chromatin to the extracellular environment studded with antimicrobial proteins to entrap and kill microorganisms. This process is distinct from apoptotic and necrotic cell death. Although the beneficial roles of NETs during infection are demonstrated, there is evidence that, if unregulated, NETs contribute to pathogenesis of several disease states.

Since their discovery, detection and quantification of NETs has mainly involved fluorescence microscopy and scanning fluorescence plate reading assays, however, they both have limitations. Therefore, we developed a novel flow cytometry assay to efficiently detect NETotic events with the use of specific NETs markers, specifically DNA/histone H1 and histone H2A. Two well established NETosis pathways were interrogated to test the sensitivity of our novel flow cytometry assay.

Following leukocyte isolation neutrophils were cultured in the presence of established NETosis stimulators/inhibitors, specifically PMA (stimulator) +/- Ro-31,8220 and DPI (inhibitors of PMA-induced NETs) or ionomycin (stimulator) +/- apamin and DNP (inhibitors of ionomycin induced NETs). After 3 hours culture, samples were fixed, blocked and stained with fluorescent antibodies for flow cytometry analyses.

Only high fluorescing double positive (DNA-H1/H2A++) events were considered as NETotic. These NETotic events were confirmed as ‘true’ NETs using an imaging flow cytometer (Image StreamX Mark II; Amnis).
The results clearly indicate that our flow cytometry assay is sensitive and reliable as it can accurately detect and quantify the different cellular mechanisms underpinning PMA- and ionomycin-induced NETs. The results obtained from our flow cytometry assay, correlate with the fluorescence microscopy and scanning fluorescence plate reading methods. By testing our flow cytometry assay for apoptotic and necrotic cell death we demonstrated that the assay is NETosis specific and therefore it overcomes the limitations of the established methods of NET quantification.

P.37 In situ immunophenotyping and identification of macrophage subpopulations in bladder cancers

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Background: Bladder cancer (BC) is UK 10th most common cancer, with about 5,300 deaths every year. To date diagnosis is only possible through assessment of the tissue obtained by invasive techniques such as cystoscopy and Transurethral Resection of Bladder Tumour. Although the efficiency and quality of the surgical techniques has improved, BC continues to have a distinctive high percentage of recurrence and progression into invasive stages.

Aim: We hypothesise that BC progression and response to treatments are influenced by the immune environment, in particular by the phenotype of macrophages in different localizations. Our aim is to identify specific macrophage markers usable in clinical histology to improve diagnostic, prediction of patient response to treatment and outcome. To this aim we phenotype individual macrophages in clinical histological sections using multiplex immunofluorescence with spectral unmixing (up to 16 markers) to obtain per cell data (markers expression, size, relative position to tumour and other cells) which combined with clinical data will allow the identification of markers of interest.

Results: Our bioinformatic analysis of the GSE32894 dataset confirmed a differential immune gene expression within BC patients. In addition, preliminary analysis of gene expression of 770 tumour and immune genes using the NanoString PanCancer IO360 in our BC samples has highlighted differences in immune and tumour signatures that do not correlate with stage or grade, but likely correlate with tumour outcome. From these studies and the literature, we have identified specific markers to test in situ.

Preliminary analysis of multiplex immunofluorescence confirmed a heterogeneity in macrophage phenotypes and suggest systematic differences between BC stages as well as between tumour-infiltrating and stromal macrophages: 95% macrophages that infiltrate Carcinoma in Situ are CD68high, CD163high, CCR2high, HO-1high, MHCIImedium-high suggesting an activated anti-inflammatory polarization stage.
P.38 SIRPα-CD47 ligation negatively regulates TLR-mediated TNFα production in macrophage subsets

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Macrophages exist on a phenotypic spectrum characterised by the polar extremes of two subsets, pro-inflammatory M1s and regulatory M2s. Both subsets express signal regulatory protein alpha (SIRPα), receptor to CD47. Characterised as a “don’t eat me” signal, SIRPα-CD47 ligation negatively regulates phagocytosis. This study investigates whether the regulatory effect of SIRPα-CD47 ligation extends to negative regulation of TLR-mediated TNFα production.

SIRPα and CD47 expression in THP-1-derived M1- and M2-like macrophages, HL-60 pro-myelocytes and K562 lymphoblasts was measured using flow cytometry. Macrophages were co-cultured with either HL-60 or K562 cells. Alone and in co-culture, macrophages were stimulated with either Staphylococcus aureus LTA, Escherichia coli K12 LPS or Porphyromonas gingivalis LPS. TNFα production was measured by ELISA.

In macrophage monoculture, all stimulation induced TNFα production, with M1s the higher producers. In all LPS co-culture conditions TNFα production was reduced compared to monoculture control, with the higher SIRPα expressing M2s experiencing more suppression. In co-culture with CD47+ HL-60 cells, M1 TNFα production was reduced by 35% for both PG-LPS (p= 3.73×10^-12) and K12-LPS (p= 2.30×10^-8). LTA-stimulated M1s were refractory. M2 TNFα production was reduced by 35% for LTA (p= 1.01×10^-8), 50% for PG-LPS (p= 1.38×10^-4) and 49% for K12-LPS (p= 8.84×10^-6). In co-culture with higher expressing CD47++ K562 cells, macrophage TNFα production was further suppressed, especially during K12-LPS stimulation where M1 TNFα production was reduced by 65% (p= 1.58×10^-14), and M2s by 73% (p= 6.32×10^-12).

These data indicate a role of SIRPα-CD47 ligation in negative regulation of TLR-mediated macrophage pro-inflammatory response, with TLR4-mediated TNFα production displaying a greater sensitivity to regulation than TLR2-mediated responses. These findings identify SIRPα as a potential therapeutic target for TNFα-mediated pathologies of uncontrolled inflammation including Crohn’s Disease and Rheumatoid Arthritis, presenting a novel mechanism for inducing functional shift from pro-inflammatory M1 towards regulatory M2 macrophage phenotype.

P.39 Characterising the resolution network in acute and age-impaired skin healing

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Efficient wound repair is essential to restore the protective barrier of the skin. Skin healing slows with age, with advanced age a risk factor for the development of chronic non-healing wounds. Age-impaired wound healing is associated with a delayed and prolonged inflammatory response. Inflammatory resolution pathways control the magnitude and duration of the inflammatory response, however the spatial and temporal expression profile of these pathways during physiological and age-impaired healing is unknown.

We made 4mm full thickness excisional wounds to the dorsal skin of young (7-9 week old), aged (12 months old) and geriatric (18 months old) male mice. Wounds were collected 1, 3, 7, 10 and 14 days
post-wounding and processed for immunohistochemistry, histology, RT-qPCR and lipidomic analysis using liquid chromatography mass spectrometry. Blood samples were also taken to identify circulating leukocyte populations.

We identified and quantified lipid resolution mediators of the docosohexaenoic acid, docosapentanoic acid, eicosapentaenoic acid and arachidonic acid bioactive metabolomes, including E and D-series resolvins, lipoxins, maresins and protectins. The expression profile of peptide and protein resolution mediators (including Annexin A1, Chemerin and alpha-Melanocyte-Stimulating Hormone) and their cognate receptors (Formyl Peptide Receptor-2, ChemR23 and Melanocortin Receptor 1) were temporally characterised during acute and age-impaired healing.

Our next steps will be to determine whether there are deficits in the resolution network in our mouse model of chronic non-healing wounds, which may contribute to repair failure. We have previously shown that we can harness specific resolution pathways to accelerate acute wound repair with reduced scarring. We will investigate whether manipulating the resolution network is sufficient to break the inflammatory cycle in chronic wounds to rescue the aberrant healing response.

P.40 Loss of splenic marginal zone B cells contributes to pneumonia susceptibility after stroke
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Post-stroke infection is the leading complication suffered by stroke patients leading to an increase in mortality and morbidity after stroke. Infections such as pneumonia are the most common and are typically acquired within the first three days of hospitalisation. Ischemic stroke is known to have suppressive effects on the immune system in both humans and experimental animal models. Reported post-stroke deficiencies in the adaptive immune system do not fully explain the rapid onset of infection described in the clinic. Marginal zone (MZ) B cells in the spleen provide rapid responses to bacterial infection using an antibody-mediated defence mechanism with the speed and low-specificity of the innate immune response. Individuals who lack a spleen due to congenital dysfunction or surgery are susceptible to the similar strains of encapsulated bacteria that typically cause infections in stroke patients. We have shown that experimental stroke in mice rapidly results in a disruption to splenic immune architecture and an extensive loss of lymphocytes and an ablation of MZ B cells resulting in deficiencies in the trapping of blood-borne antigen and reduced levels of circulating IgM. Circulating IgM levels were similarly suppressed in acute stroke patients. Spontaneous bacterial infection, correlating with the severity of brain injury, occurs in these animals. Adrenergic signalling mediates these deficits suggesting the involvement of autonomic pathways in brain-immune communication affecting systemic B cell function after stroke. These novel findings suggest that the loss of innate-like functions of B cells after stroke is an important determinant of susceptibility to infection and highlight this pathway as an important target for intervention.
Rapid clearance of cellular debris by microglia limits secondary neuronal cell death after brain injury in vivo

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Moderate or severe traumatic brain injury (TBI) causes widespread neuronal cell death, which occurs in two phases. Primary cell death is a direct and immediate consequence of physical injury, whereas secondary cell death occurs in the hours and days after the initial insult as a result of complex neurotoxic processes. Microglia, the resident macrophages of the brain, react to brain injury by migrating to the lesion site, where they take up neuronal debris through phagocytosis. Microglial phagocytosis after brain injury can have both beneficial (e.g. debris clearance) and detrimental (e.g. respiratory burst, phagoptosis) consequences. Hence, whether the overall effect of microglial phagocytosis after brain injury in vivo is neuroprotective or neurotoxic is not known. Here we capitalise on the amenability of larval zebrafish for live imaging to establish a system with which to carry out dynamic real-time analyses of the mechanisms regulating secondary cell death after brain injury in vivo. We show that mechanical injury to the larval zebrafish brain induces distinct phases of primary necrotic and secondary apoptotic cell death. Secondary cell death is decreased when excitotoxicity is pharmacologically reduced, reflecting findings from mammals. Microglia arrive at the lesion site within minutes of injury, where they rapidly engulf primary necrotic cells. Importantly, the rate at which apoptotic cells appear during the phase of secondary cell death is increased when the rapid removal of primary necrotic cells by microglia is pharmacologically or genetically impaired. In summary, our results provide evidence that microglial debris clearance is neuroprotective after mechanical brain injury in vivo.

Fatty acid oxidation can drive human monocyte derived CCL20 in the RA synovial environment

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Metabolic pathways are now considered to have a governing role in myeloid cell phenotype and function. This is particularly evident in murine macrophages where glycolysis and fatty acid oxidation (FAO) have been implicated in inflammatory cascades and immune regulation respectively. However, investigation of human monocyte metabolism in the context of the hypoxic and inflammatory rheumatoid arthritis (RA) synovium is lacking.

Therefore, we emulated the hypoxic RA environment in vitro and metabolically profiled human monocytes by LC-MS metabolomics. Additionally, we assessed if altered metabolic pathways have a functional impact on monocytes under disease-relevant conditions.

We illustrate that hypoxia significantly enhances monocyte-derived CCL20 & IL-1β in response to LPS stimulation. Metabolomic analysis showed that hypoxia increases glycolytic intermediates at the expense of carnitine metabolites, suggesting that hypoxia limits FAO. Interestingly, studies to pharmacologically inhibit (etomoxir) or enhance FAO (carnitine media supplementation) revealed a novel role for FAO in the production of CCL20.
In the context of RA, transcriptomic analysis of matched ex vivo RA blood monocytes and synovial fluid (RA-SF) macrophages proposed that monocyte infiltration into the RA milieu alters fatty acid metabolism and augments CCL20 expression. In support, in vitro stimulation of monocytes with RA-SF in hypoxic conditions enhanced carnitine abundance and CCL20 production. Furthermore, CCL20 release after RA-SF stimulation was significantly increased by carnitine media supplementation.

This work has identified a novel inflammatory mechanism in RA which links FAO to CCL20 production in human monocytes. This may contribute to RA disease pathogenesis by promoting the recruitment of Th17, B cells and osteoclastogenesis.

P.43 IFN-γ and IL-17A differentially influence the response of human macrophages and neutrophils to Pseudomonas aeruginosa infection

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Macrophages are important orchestrators of inflammation during bacterial infection acting both as effector cells and as regulators of neutrophil recruitment and life span. Differently activated macrophage populations with distinct inflammatory and microbicidal potential have been described. Our previous work unveiled a positive and a negative correlation between levels of IFN-γ and IL-17A, respectively, and lung function in cystic fibrosis, particularly in patients chronically infected with P. aeruginosa. This study sought to define key parameters in human anti-bacterial immunity under Th1- and Th17-dominated inflammatory conditions; the final aim was to identify unique characteristics that could be fine-tuned therapeutically to minimise tissue damage while maximising bacterial clearance. Towards this aim neutrophils were incorporated into cultures of macrophages treated with IFN-γ or IL-17A and infected with P. aeruginosa. The intent of this design was to model (i) initiation of inflammation by infected macrophages and (ii) delayed arrival of neutrophils and their exposure to macrophage-derived cytokines. Under these conditions IFN-γ decreased bacterial killing and promoted production of the monocyte chemoattractant MCP-1. In contrast, IL-17A promoted bacterial killing but did not affect MCP-1 production. Secretion of the pyrogen IL-1β was significantly lower in the presence of IFN-γ compared to IL-17A and correlated with levels of IL1B transcript in infected macrophages.

P.44 Prostaglandin E2 promotes intestinal inflammation via inhibiting microbiota-dependent regulatory T cells

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Intestinal homeostasis and disease are regulated by the gut microbiota in part through mechanisms that involves modulation of regulatory T cells (Tregs), yet the environmental cues that exert physiological control over microbiota-Treg crosstalk are incompletely defined. Non-steroidal anti-inflammatory drugs (NSAIDs) that inhibit prostaglandin E2 (PGE2) production are generally avoided in
gut inflammation because of their adverse effects to the intestinal barrier. However, polymorphisms in the PTGER4 gene (encoding the PGE2 receptor EP4) are associated with intestinal EP4 overexpression and a more severe disease phenotype in patients with inflammatory bowel disease (IBD). While the protective action of PGE2 on the gut barrier is well recognized, whether PGE2 has potentially noxious functions on intestinal inflammation and the underlying mechanisms remain unstudied. Here, we report that PGE2 promotes intestinal inflammation by inhibiting mucosal Tregs in a manner that is dependent on the gut microbiota. Blockade of endogenous PGE2 induces anti-inflammatory microbial shifts, e.g. increase in short chain fatty acid-producing commensal bacteria. Transfer of intestinal microbiota from mice that have been treated with NSAIDs attenuates intestinal inflammation. Mechanistically, PGE2-microbiota interaction regulates intestinal mononuclear phagocytes (MNPs) and type I interferon signaling. Depletion of intestinal CD11b+ MNPs or deficiency of interferon-α/β receptor diminishes PGE2-dependent Treg inhibition. Furthermore, negative correlations between the PGE2 pathway and Treg signature gene expression are observed in human colon biopsies from patients with IBD. Taken together, our findings, for the first time, provide evidence for PGE2-mediated disruption of microbiota-Treg communication that promotes intestinal inflammation, indicating that NSAIDs may be beneficial for certain subtypes of IBD such as T cell-mediated intestinal inflammation.

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P.45 Heligmosomoides polygyrus infection induces anti-viral gene expression in the lung epithelium and immune cells

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Infant respiratory viral infections are a major cause of infant hospitalisation and a risk factor in the development of persistent wheeze, airway allergic responses and ultimately asthma. We have recently shown that ongoing infection in mice with the gut helminth Heligmosomoides polygyrus protects against respiratory syncytial virus (RSV) infection, reducing viral load, associated immunological changes and airway impairment. This protective effect is dependent upon the induction of type-I interferons in the gut and/or the lung and the presence of normal gut microbiota. We now demonstrate that intravenous serum transfer from mice 10 days after H. polygyrus infection to naive mice induced similar increases in interferon beta and interferon stimulated gene expression as seen in H. polygyrus infection, and reduced peak viral load after subsequent RSV infection. This induction of anti-viral genes is observed across both the lung epithelial cells and immune populations including interferon beta positive lung macrophages. We hypothesised that this antiviral myeloid state originates systemically and found elevated interferon beta levels in the bone marrow of H. polygyrus infected animals. Furthermore, the bone marrow exhibited elevated myelopoiesis driving an increase in circulatory monocyte populations and in recruited monocytes in the lung. These results suggest that during H. polygyrus infection serum borne factors induce an anti-viral state in the lung epithelium and circulatory monocytes allowing these cells to mount a rapid and protective response to RSV-infection.
**Neutrophils in advanced non-small cell lung cancer**

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**BACKGROUND**

Raised peripheral blood neutrophil counts are known to correlate with worse patient outcomes in cancer. Neutrophils have also been shown to be present in the tumour microenvironment, although their function with respect to primary and metastatic tumour progression remains poorly defined. Given the heterogeneity of neutrophil populations described in this setting, we aimed to characterise the phenotype and function of neutrophil subpopulations present in advanced non-small cell lung cancer (NSCLC), and assess whether the metastatic niche influences neutrophil function. Through better understanding of these subpopulations, and the impact of the local tissue environment upon their function, new therapeutic targets may be identified.

**METHODS**

Neutrophils were extracted from the blood and pleural fluid (metastatic site) of patients with advanced NSCLC. Subpopulations were defined by density, morphology and surface marker expression (flow cytometry). Neutrophils were also extracted from the blood of healthy donors. Ex vivo studies of neutrophil function were carried out in conditions simulating the metastatic niche (e.g. in the presence of pleural fluid supernatant).

**RESULTS**

There is an expanded population of CD66b+CD11b+CD15highCD14−CD49d− immature low-density neutrophils in advanced NSCLC. Blood neutrophils of patients with NSCLC have a survival advantage ex vivo in culture when compared to those of healthy donors. Pleural fluid supernatant manipulates the apoptosis phenotype of healthy donor neutrophils. Furthermore, pleural fluid supernatant conditions healthy donor neutrophils to suppress CD8 T cell proliferation.

**CONCLUSIONS**

Several neutrophil subpopulations are present in advanced NSCLC. Our data suggests that neutrophils at the metastatic site may be longer lived and immunosuppressive.

**Characterising the effects of extracellular Annexin A1 on macrophage phenotype and epithelial cell function in lung adenocarcinoma**

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Purpose of the study: In lung adenocarcinoma increased AnnexinA1 (ANXA1) expression within tumour cells is associated with metastatic spread and poor prognosis. Present both as an intracellular and secreted factor, its potentially pleotropic roles in influencing the tumour microenvironment are poorly characterised. Tumour-associated macrophages (TAMs) contribute towards tumour growth and metastasis through their pro-resolution phenotype. ANXA1 accelerates inflammation resolution but its role in tumour progression is less well understood. We hypothesised that extracellular ANXA1 released by lung adenocarcinoma cells would drive TAMs towards a pro-resolution phenotype as well as influencing epithelial cell function. These could therefore serve as potential mechanisms by which the detrimental effects of ANXA1 are mediated.
Methods: Monocyte-derived macrophage (MDMs) were generated and cultured from peripheral blood of healthy human volunteers before treatment with ANXA1. Effects on macrophage surface marker expression and phagocytosis of apoptotic cells as well as lung epithelial cell proliferation and migration were examined.

Results: Within the parameters of the in vitro systems used, we were unable to demonstrate any effect of extracellular ANXA1 on macrophage surface marker expression or phagocytic capacity. Additionally, ANXA1 inhibited both epithelial cell proliferation and migration in a concentration-dependent manner.

Conclusions: Further work, including characterisation of other aspects of macrophage function, are required to confirm whether ANXA1 plays a role in altering macrophage phenotype. The observation that exogenous ANXA1 inhibits epithelial cell proliferation and migration suggests that if ANXA1 directly contributes to tumour progression it may be through intracellular interactions rather than extracellular ANXA1 binding to surface receptors.

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P.48 Investigating the role of TREM2 in an experimental model of chronic cerebral hypoperfusion
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Chronic cerebral hypoperfusion (CCH) resulting from cerebrovascular disease is a key contributor to white matter damage, cognitive impairment and dementia. Although the precise mechanisms remain unclear, neuroinflammation is suggested to mediate CCH-induced white matter damage and cognitive impairment. Neuroinflammation is observed in various neurological conditions and is implicated in both the initiation and progression of dementia. Mutations in the microglial immunoreceptor triggering receptor expressed on myeloid cells 2 (TREM2) increase susceptibility to neurodegenerative conditions providing key evidence for the role of neuroimmune dysfunction in dementia pathogenesis. Precisely how TREM2 dysfunction contributes to neurodegeneration and dementia remains to be established however, particularly in relation to cerebrovascular causes of degeneration.

To investigate the role of TREM2 in cerebrovascular-mediated white matter damage, the bilateral common carotid artery stenosis (BCAS) model of CCH was implemented in WT and TREM2-/ C57BL/6Ntac mice. Twenty eight days after surgery, mice demonstrated diffuse white matter damage associated with activated microglia. In the absence of TREM2 expression, white matter damage was exacerbated yet microgliosis was blunted. Peripheral leucocyte recruitment was also reduced in the absence of TREM2 expression suggesting TREM2 plays a key role in regulating neuroinflammatory responses to cerebrovascular dysfunction. As TREM2 deficiency exacerbated white matter damage it could be postulated TREM2-regulated processes play a key role in the maintenance of myelin integrity. However, the precise mechanisms remain unclear. Furthermore, it remains unclear if TREM2 deficiency impacts white matter function or cognition. Future studies will therefore determine the impact of TREM2 deficiency on white matter function in response to chronic cerebral hypoperfusion. Studies investigating the impact of cerebral hypoperfusion on the microglial transcriptome will also advance our understanding of microglial responses to cerebrovascular dysfunction and may reveal novel TREM2-regulated pathways which attenuate white matter damage and cognitive impairment.
Neutrophils are the most abundant leucocyte and a key first responder at sites of injury and infection where they are responsible for host defence and repair. Being predominantly glycolytic, neutrophil metabolism has uniquely adapted to support function in sites where oxygen is limited. There is increasing evidence in other cells that metabolic fate governs cellular function, however, how the availability of nutrients other than oxygen regulates neutrophil metabolism and function remains poorly understood. Moving from a glucose replete, to deplete environment during diapedesis, we hypothesised that glucose availability acts as a metabolic controller, modifying neutrophil behaviour. Through ex vivo culture of both human blood and murine inflammatory neutrophils, we show that glucose availability regulates neutrophil survival. To establish the metabolic basis for this response, we cultured human blood neutrophils in a glucose replete or deplete environment before performing extracellular flux analysis (Seahorse). Confirming previous reports, neutrophils were principally glycolytic with glucose availability having no effect on their ability to consume oxygen. Surprisingly, glucose deprivation in culture established a marked increase in the glycolytic capacity of neutrophils, raising the possibility that they have the capacity to use alternative substrates to maintain glycolysis when glucose is deficient. It is well established that neutrophil activation is associated with an increased flux through glycolysis and we hypothesise that glucose deprivation may act as a danger-associated signal, mediating their activation at appropriate sites. Further work will examine how the supply of excessive glucose, such as in states of diabetes mellitus, disrupts this physiological response to the detriment of host defence.

P.50 Exploring the pro-resolving characteristics of insoluble immune complexes in human neutrophils
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Background:
Immune complexes (ICs) are antigen-antibody aggregates that can activate neutrophils and induce a range of functions such as phagocytosis, generation of reactive oxygen species (ROS), production of cytokines and release of inflammatory mediators. Three types of ICs can be present in inflammatory conditions in the human body, soluble ICs, insoluble immune complexes (iIC) and immobilised ICs. Phagocytosis-induced cell death (PICD) is a well-established phenomenon where internalisation of pathogens induces neutrophil apoptosis, promoting the resolution of inflammation. Our previous findings showed that iICs induce neutrophil apoptosis via a non-canonical pathway; hence we investigated whether iIC-induced neutrophil apoptosis is a form of PICD.

Material and methods:
Freshly isolated neutrophils from healthy donor blood were stimulated with iICs or particles (IgG-opsonised zymosan or latex beads). (i) Induction of apoptosis was assessed at different time-points (0h, 3h, 6h, 9h, 12h and 24h) by flow cytometry and morphological analysis. (ii) Immunofluorescence
was used to investigate internalisation of ingested particles. (iii) IgG degradation of internalised iICs and latex beads was analysed by Western Blot.

Results:
We show that (i) iICs and zymosan induce neutrophil apoptosis but IgG-opsonised latex beads do not. (ii) Internalisation of beads and iICs depends on different signalling pathways and regulators, and the rate of internalisation is different. (iii) Both internalisation events trigger IgG degradation. Chloroquine, an endocytosis blocking agent, prevents iIC degradation but not the degradation of IgG from latex beads.

Conclusions:
iIC-induced neutrophil cell death and PICD are mechanistically distinct. Internalisation and degradation of iICs and opsonised latex beads are regulated by separate signalling pathways. iIC-induced neutrophil apoptosis might play a major role in the resolution of inflammation in autoimmune diseases.

P.51 The role of inflammasomes and IL-1beta in sterile CNS damage and regeneration
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Inflammasomes are cytosolic protein complexes that sense sterile or non-sterile danger signals. Their role is to alert the immune system by generating pro-inflammatory IL-1beta and IL-18. Myelin damage (demyelination) is a hallmark of multiple sclerosis an uncurable inflammatory disease of the central nervous system (CNS) that has recently been associated with inflammasome activity. Because inflammasomes are implicated in both tissue destruction and in the response to tissue damage this study aims to determine if inflammasome activity is a component of the local tissue response to myelin damage and the potential implications for myelin regeneration.

Inflammasome expression was analysed in murine CNS tissue after demyelination. For functional studies of inflammasome activity in de- and remyelinated tissue ex vivo brain slices and in vitro oligodendroglial cultures were used.

NLRP3, AIM2, NLRC4, ASC and caspase-1, as well as inflammasome product IL-1beta, were present in demyelinated CNS lesions including in remyelinating lesions demonstrating the functional significance of inflammasome components in response to myelin damage. As glial cells express IL-1 receptors and can therefore respond to inflammasome activity, we analysed the effect of IL-1beta on myelin producing oligodendrocytes. We observed enhanced oligodendrocyte proliferation, myelin protein production and myelination of axons with inflammasome product IL-1beta thus linking an innate immune complex to a developmental and regenerative program in the CNS.

In summary, we show that inflammasome components are detected in lesions after CNS demyelination suggesting a key role for inflammasomes and its mediators in the CNS tissue response to myelin damage. Ex vivo, inflammasome product IL-1beta boosted oligodendrocyte differentiation and myelin production indicating a beneficial effect for myelin regeneration that could have implications for new therapeutic approaches in multiple sclerosis.
Investigating the phenotypic changes that occur in microglia and macrophages in response to intracerebral haemorrhage and whether they can be modified to improve outcome

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With a 40% fatality rate within the first month, and two-thirds of survivors with permanent disability, intracerebral haemorrhage (ICH) is the deadliest subtype of stroke. ICH shares important aetiological mechanisms with vascular dementia, notably cerebral small vessel disease, and risk of dementia is doubled in stroke patients.

While the initial haematoma physically disrupts the cellular architecture; toxic blood components and other stimuli subsequently trigger an inflammatory response influencing secondary brain injury. This inflammatory response, characterised by activated microglia and the infiltration of peripheral myeloid cells, may have both detrimental and beneficial actions, by affecting haematoma clearance, as well as the production of pleiotropic inflammatory mediators.

The aim of this project is to understand the wide-array of phenotypic changes that occur in microglia and macrophages in response to ICH. These are currently not well understood but are imperative to understand so that immunomodulatory approaches can be optimally designed.

We will use the extensive resource of post-mortem brain tissue from ICH cases in the Edinburgh Brain Bank to define the spatio-temporal changes in the CNS myeloid cell profile. With over 130 ICH cases both with and without underlying small vessel disease, a detailed evolution of the neuroimmune response to ICH will be determined and key measures correlated with important clinical variables. In complementary preclinical studies enabling intervention approaches, we will augment CNS myeloid cell accumulation using our recently validated approaches in a mouse model of intracerebral haemorrhage to determine effects on haematoma pathology and functional outcome after ICH.

These studies will provide new insights into how CNS myeloid cells react to ICH and provide proof of concept for a novel approach to manipulate these cells for improving outcome after ICH. Current studies will focus on acute and sub-acute outcomes however we envisage future work will examine longer-term cognitive and behavioural performance.

Stratifying Idiopathic Pulmonary Fibrosis patients through iron metabolism in macrophages

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Idiopathic Pulmonary Fibrosis (IPF) is a severe progressive lung disease with unknown etiology and limited therapeutic options. Alveolar macrophages (AM) have been implicated in the pathogenesis of IPF as they are key in homeostatic mechanisms in the lung and in the development of pathologic fibrosis and inflammation. Longitudinal studies carried out in our group have demonstrated that differential AM expression of CD71 (the transferrin receptor, Tfr1), CD206 and CD163 (the scavenger receptor for haemoglobin-haptoglobin complexes) are associated with different rates of progression. IPF patients with CD71highCD206highCD163low-expressing macrophages have a significantly poorer survival than CD71lowCD206lowCD163high-expressing patients. This suggests an implication of iron metabolism in the pathogenesis and progression of disease.
We hypothesised that changes in iron metabolism in AM could be a tractable marker of disease progression and aim to develop a ‘smart-fluorophore’ which could be used to visualise and quantify iron stores in live macrophages.

Our preliminary data show that patients’ AM incubated with a fluorescent iron probe show different levels of iron depending on the presence or absence of a chelator. This proof-of-concept experiment lays the basis for the optimisation of the probe in a live highly auto-fluorescent context. Current efforts are focused on increasing the number of patients tested with two probes in vitro, in conjunction with other laboratory techniques, in order to assess iron in macrophages.

P.54 A novel mouse model for pre-clinical in vivo evaluation of immuno-modulatory therapy in Malignant Pleural Mesothelioma

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Malignant Pleural Mesothelioma (MM) is a uniformly fatal cancer caused by asbestos exposure. It is characterised by a highly pro-inflammatory microenvironment, which contributes to tumour initiation and progression.

MPM does not respond to conventional therapies and lacks the common driver mutations that have permitted targeted therapeutics in other cancers. Alternative MPM treatments are thus urgently required. Immuno-modulatory strategies, which alter the behaviour of certain immune cell populations in the tumour microenvironment, have shown remarkable effects in other cancers and offer a conceivable route to improved MPM outcomes.

Current MPM mouse models to interrogate this phenomena are unfit for purpose and hampered by long latency (in asbestos only models) and variable penetrance/off target effects in existing transgenic models.

We describe our novel mouse model that combines intrapleural injection of asbestos with site-specific deletion of the key tumour-suppressor genes (NF2, CDKN2A, BAP1) that are lost in human MPM. This is the first physiologically intact and anatomically accurate model of MPM that combines asbestos-driven chronic inflammation with loco-temporal deletion of relevant genes and is a unique platform to investigate early and late stage events in the inflammatory evolution of MPM.

We have identified a key immune related protein (CD98hc), which is overexpressed in MPM and tumour associated macrophages. We have shown in patient tissue microarray that CD98hc expression status is a negative clinical prognostic factor and in xenograft MPM models that loss of CD98hc attenuates tumour growth.

Although clinical trials of immune modulation in MPM are already underway, poor response rates (around 20%) suggest that combination therapy is likely to be required. We will utilise our novel mouse model to determine the likelihood of success of several immune modulation strategies, including CD98hc inhibition, (+/- existing therapies) in vivo, offering a rational and rapid platform for testing therapeutic approaches prior to clinical trials.
Investigating the influence of TREM2 on CNS myeloid cell reactivity and resolution of acute brain inflammation

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Microglia, the resident immune cells of the central nervous system (CNS), fulfill essential roles in the maintenance of a healthy brain parenchyma and mediate innate neuroimmune activity. Gene variants of TREM2, an immunoglobulin receptor expressed on microglia, are associated with chronic neurodegenerative conditions including Nasu-Hakola disease, Alzheimer’s disease, Parkinson’s disease and frontotemporal dementia. Accordingly, it is hypothesised TREM2 contributes to microglial regulation of brain homeostasis. Growing evidence also suggests TREM2 regulates the magnitude and profile of microglial responses to inflammatory and injurious insults.

This project aims to elucidate the role of TREM2 in microglial reactivity and the recruitment and phenotypes of CNS myeloid cell populations in response to acute inflammatory insult. The project incorporates in vitro assessments of TREM2-mediated responses; primary murine microglial and macrophage cultures isolated from wild-type and TREM2 KO mice are challenged with inflammatory stimuli to identify shifts in pro-inflammatory and pro-resolving phenotypes. Furthermore, current work is using a recently-validated in vivo model of self-limiting acute intracerebral inflammation to assess the role of TREM2 in mediating resolution of inflammation with a focus to alterations in the temporal composition and kinetics of myeloid cell influx and myeloid cell-to-cell interactions.

Overall, we anticipate this project will provide insight to the influence of TREM2 in controlling an appropriate neuroinflammatory response relevant to maintaining a healthy brain over the life-course. More broadly, this work should inform on strategies to manipulate TREM2-dependent pathways therapeutically that are relevant for both acute and chronic neurological conditions that may benefit from modulating resolution of harmful inflammation.

Apolipoprotein A-IV exhibits anti-inflammatory properties in vitro and is decreased in allergic patients

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Background. Recent studies pointed to a crucial role for apolipoproteins in the pathogenesis of inflammatory diseases. However, the role of apolipoprotein-IV (ApoA-IV) in allergic inflammation has not been addressed thus far.

Objective. Here we explored the anti-inflammatory effects and underlying signaling pathways of ApoA-IV on eosinophil effector function in vitro and in vivo.

Methods. Migratory responsiveness, Ca2+-flux and respiratory burst of human peripheral blood eosinophils were assessed in vitro. Monocyte/macrophage function and polarization were explored by flow cytometry and mediator release was measured by ELISA. Allergen-driven airway inflammation was assessed in a mouse model of acute house dust mite-induced asthma. ApoA-IV serum levels were determined by ELISA.

Results. Recombinant ApoA-IV potently inhibited eosinophil responsiveness in vitro as measured by Ca2+-flux, shape change, integrin (CD11b) expression and chemotaxis. The underlying molecular
mechanism involved the activation of Rev-ErbA-α and induced a PI3K/PDK1/PKA-dependent signaling cascade. ApoA-IV also inhibited integrin expression in monocytes and macrophages, enhanced PGE2 release from monocytes, curbed TNF-α synthesis in macrophages and directed macrophage polarization towards the anti-inflammatory M2 type. Systemic application of ApoA-IV prevented airway hyperresponsiveness (AHR) and airway eosinophilia in mice following allergen challenge. ApoA-IV levels were decreased in serum from allergic patients compared to healthy controls.

Conclusion. Our data suggest that ApoA-IV is an endogenous anti-inflammatory protein that potently suppresses effector cell functions in eosinophils, monocytes and macrophages. Thus, exogenously applied ApoA-IV may represent a novel pharmacological approach for the treatment of allergic inflammation and other eosinophil-driven disorders.

P.57 The effect of Prostaglandin E2 on Interleukin 22 production by CD4+ lymphocytes
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The pathogenic mechanisms involved in the development of common human inflammatory conditions such as eczema are not fully understood resulting in often limited targeted treatment options for some of these diseases. The Prostaglandin E2 (PGE2)/Interleukin 22(IL-22)/Interleukin17(IL-17) pathway has been shown in murine models to play an important role in autoimmune and inflammatory diseases including chronic skin inflammation in mouse models of allergic contact dermatitis. The PGE2/IL-22 axis has not been studied in man however. Therefore we investigated this pathway in healthy humans firstly. We also analysed both PGE2 and IL-22 pathway gene expression in a range of diseases including eczema and psoriasis to determine if these were upregulated. We also determined whether there was a positive correlation between PGE2 and IL-22 pathway gene expression in a range of inflammatory, neoplastic and infectious diseases.

Methods:
CD3+ T cells were isolated from healthy whole blood and cultured in IL22 and IL17 priming conditions with or without PGE2 to study its effect on the percentage of IL-22 and IL-17 producing CD4+ lymphocytes. Using a bioinformatics approach we mined datasets in order to determine whether PGE2 and IL-22 pathway genes are correlated.

Results:
Fluorescence-activated cell sorting demonstrated that PGE2 significantly promoted increased differentiation into IL-22 and IL-17-producing CD4+ T cells compared with medium (-IL23 -IL18), vehicle (+IL23+ IL18) and control/cytokines (+IL23 +IL18). This effect was not seen for CD4 T cells. Our gene data mining study indicates a strong positive correlation between the expression of PGE2 signaling genes and IL-22 signaling genes in not only inflammation but also infection and cancer in man.

Conclusion:
Our work strongly suggests that PGE2 signaling promotes IL-22 production by T cells in vitro and our gene mining data indicates that this pathway may play a key role in a host of human diseases.
Tenascin-C (Tnc) is an ECM glycoprotein implicated in the pathogenesis of a variety of chronic inflammatory diseases. At sites of inflammation Tnc becomes highly upregulated where it acts to create a local pro-inflammatory ‘niche’ via stimulation of the TLR4 receptor on stromal and immune cells. Inflammatory Bowel Disease (IBD) is a chronic inflammatory disease of the large intestine with varied aetiology and presentation in patients. Tnc has been implicated in IBD with increased levels observed in the inflamed mucosa and serum of patients. However, the exact role Tnc may be playing in the disease has yet to be fully investigated.

Acute colitis was chemically induced in mice by administration of 2-3% Dextran Sulphate Sodium (DSS) in their drinking water. Tnc was found to be significantly upregulated in the colons of DSS treated mice at the expression and protein level. Additionally, this was concurrent with the upregulation of other key pro-inflammatory factors such as IL-6, TNFα, and IL-1β. Immunohistochemistry revealed that Tnc is basally expressed in the colon, however, upon induction of colitis it is significantly upregulated in the mucosa appearing to co-localise with areas of ulceration. Co-staining with immune cell markers has shown that immune infiltration coincides with Tnc upregulation. Subsequent studies in Tnc-/- mice have demonstrated a protective effect of Tnc’s genetic ablation with gross and histological disease parameters reduced in comparison to wild-type controls.

Studies have previously demonstrated the role of Tnc in maintaining aberrant inflammatory responses, such as antigen-induced arthritis, with a protective affect observed upon Tnc ablation. This study further supports these findings in an IBD pre-clinical animal model and suggests Tnc may be playing a similar pro-inflammatory role. This suggests that therapies targeting Tnc may also benefit patients suffering from IBD. Further study of the exact role and best methods to target Tnc are warranted.

Human Rhinovirus 16 infection of human macrophages impairs their clearance ability by perturbing phagosome maturation

Introduction and hypothesis
Chronic Obstructive Pulmonary Disease (COPD) is characterised by increased numbers of alveolar macrophages (AM) with diminished phagocytic function. This could drive exacerbations where human rhinovirus (HRV) is frequently isolated. We hypothesised that HRV infection of macrophages was driving this phagocytic impairment.

Methods
AM or monocyte derived macrophages (MDMs) were challenged with HRV16 and the endocytic machinery analysed by microscopy. We also analysed the recruitment of endocytic markers around phagosomes in infected macrophages following HRV16 infection and a second trigger. Finally, we
quantified the expression and localisation of these markers in infected macrophages by western blotting and flow cytometry.

Results
We found endocytic markers were mislocalised with altered expression profiles in infected macrophages and not properly recruited around phagosomes, impairing phagosome maturation and bacterial clearance. Reactive oxygen species (ROS) and hydrolase production was impaired in infected macrophages also perturbing bacterial clearance. Lastly, microtubule dynamics were affected in infected macrophages impairing clearance further. We are currently using in depth cell biology to explore host proteins we believe HRV16 affects driving these defects.

Conclusion
Our results demonstrate HRV16 impairs intracellular clearance in macrophages by perturbing the endocytic machinery and affecting microtubule dynamics. They could explain why COPD patients show virus induced exacerbations and secondary bacterial infections. Further analysis of how HRV16 hijacks these pathways offers the potential to design novel therapeutics to treat COPD exacerbations.

P.60 Defining the Role of Neutrophils in Acetaminophen-Induced Liver Injury and Regeneration

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Neutrophils, essential innate immune cells, often have a deleterious role in sterile inflammation, with dysregulated effector functions and an extended life span. Neutrophils also assist resolution by clearing debris, ‘informing’ phagocytes, promoting revascularization and co-ordinating repair. It is unknown if neutrophils have damaging actions or are resolving and reparative in acetaminophen toxicity, a leading cause of liver injury-induced death in Western world countries. It is also unknown if neutrophils can be modulated to improve hepatic repair following acetaminophen toxicity. We hypothesise that neutrophils contribute to acetaminophen-induced hepatic injury and triggering neutrophil apoptosis could improve hepatic repair.

Here we show the preliminary results from optimising a mouse model of acetaminophen toxicity and various methods to investigate the neutrophil in vivo. We also show that a cyclin-dependent kinase inhibitor, AT7519, given at peak hepatic neutrophil infiltration, induces hepatic neutrophil apoptosis. This neutrophil apoptosis is associated with evidence of increased hepatic damage; alanine aminotransferase, aspartate aminotransferase (p <0.005) and percentage weight loss increase (p <0.05).

Neutrophils have been shown to be important for tissue repair, so it is possible that the loss of neutrophils following AT7519 is detrimental to hepatic regeneration. Other possible mechanisms require investigation, including saturated efferocytosis and neutrophil secondary necrosis, or the action of AT7519 on other cell types. Defining the role of neutrophils in acetaminophen toxicity and their mechanisms of action may provide insight into new treatments for this common and fatal toxicity. Additional methods to manipulate the neutrophil infiltration, life span and subsequent clearance by efferocytosis are underway.
Investigating the role of inflammasomes in tissue repair

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Inflammasomes are cytosolic multi-protein complexes that assemble in response to various danger stimuli. Activated inflammasomes process pro-IL-1beta and pro-IL-18 into their bioactive forms, which play a role in promoting tissue regeneration. For instance, inflammasome-derived IL-1beta (IL-1β) promotes TGF-beta secretion in wound healing thereby initiating epithelial cell and fibroblast proliferation. Inflammasomes might also be decisive drivers in early lung tissue regeneration by activating and recruiting cells required for efficient tissue repair. The lung epithelial cell precursors and stem cell niches possess reparative capacity after injury. However, the exact mechanisms of inflammasomes in lung tissue repair are unclear.

We hypothesise that inflammasome activity supports tissue regeneration after lung injury. The aim of this study was to determine which inflammasomes are expressed in lung homeostasis and damage by using a model of acute respiratory distress syndrome (ARDS). Furthermore, we investigated if inflammasome deficiency affected damage and repair.

LPS challenge caused pathologic lung damage as shown by H&E. Lung injury score of LPS treated mice was significantly increased on day 1 and day 3 but reverted to nearly normal on day 7. Preliminary findings revealed that inflammasome markers NLRC4, AIM2 and ASC were found expressed in healthy murine lung tissue. CD45+ immune cells and CD68+ macrophages were the predominant cell types expressing inflammasome markers in the healthy lung. LPS treatment induced a significant decrease in body weight in both C57BL/6 and NLRP3−/− mice after 24 hours. LPS challenge induced ASC, NLRC4, AIM2 and NLRP3 and pro-IL1β expression after 24hrs post-treatment indicating a potential role of these inflammasome proteins in the response to tissue damage. Future studies will reveal the impact of inflammasome activity on lung tissue repair in vivo and lung progenitor cells in vitro. The outcome of this study might uncover potential novel therapeutic targets for diseases such as ARDS.
with apoptotic cells, we showed that phospho-STAT3 (pSTAT3) is activated upon phagocytic cargo digestion, and is required to maintain efficient phagocytosis. We confirmed our findings in vivo, using a mouse model of ALI in the presence of the phagocytic cell tracer, PKH26PCL. Further, we hypothesised that the mechanism underlying the pro-phagocytic function of pSTAT3 was in activating autophagy, allowing macrophages to efficiently process damaged organelles from ingested cells to maintain efficient phagocytic function. To test this hypothesis, we used in vitro phagocytosis assays followed by qPCR analysis and transmission electron microscopy of phagocytosing macrophages in the presence or absence of a pSTAT3 inhibitor. Inhibiting pSTAT3 stifled the induction of phagocytosis-induced autophagy-related genes, such as Ambra1 and Ulk1. Moreover, numbers of late auto-vesicles were reduced in pSTAT-3 inhibited macrophages. IL6 stimulates phosphorylation of STAT3, and its addition to in vitro phagocytosis assays rescued the autophagy-pathway inhibition induced by pSTAT3 inhibition. In conclusion, we demonstrated that pSTAT3 activation sustains phagocytosis in macrophages through the activation of autophagy thereby improving their ability to process damaged organelles ingested through phagocytosis. Future work will elucidate if and how pSTAT3 can be targeted in macrophages to improve liver regeneration.

P.63 IgM is required for IL-4 dependent expansion of macrophages in the pleural cavity

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Site specific proliferation of tissue resident macrophage populations is an important feature of type 2 immune responses. Components of the complement cascade have recently been shown to play a critical role in potentiating macrophage proliferation in the peritoneal cavity and liver. Here we demonstrate that IgM is required for the expansion of macrophages in the pleural cavity during type 2 immune responses. Using a mouse model in which there is no secreted IgM we found that pleural macrophages failed to increase in number in response to IL-4 complex. RNAseq analysis of IgM sufficient and deficient pleural cavity F4/80hi macrophages revealed impaired activation of genes involved in DNA damage repair and cell cycle checkpoint progression following in vivo exposure to IL-4. In addition, we found that in the absence of secreted IgM, pleural macrophages failed to increase oxidative respiration in response to IL-4. Using FcμR−/− mice, we ruled out the possibility that IgM directly activated macrophages via the specific IgM receptor FcμR. Finally, we confirmed the importance of IgM regulation of pleural macrophage expansion using infection with the tissue tropic filarial worm Litomosoides sigmodontis, a parasite which simultaneously expands pleural macrophages and activates the local secretion of IgM by fat-associated lymphoid cluster B cells. In the absence of IgM, reduced macrophage number correlated with reduced killing of L3/L4 stage larvae within the pleural space and reduced killing of L3 larvae in vitro. Ongoing experiments are testing the hypothesis that the macrophage changes observed in the absence of secreted IgM are due to tissue specific regulation of the complement cascade.
**P.64 Control of post prandial cholesterol level by Tim4+ resident adipose tissue macrophages**

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Chronic exposure to excess fat leads to dysfunction of adipose tissue and obesity. This is characterized by an important recruitment of inflammatory macrophages associated with low-grade inflammation and insulin resistance. While the role of these macrophages attracted a lot of attention, the origin and role of adipose tissue macrophages (ATM) in the lean state have not been studied. Genome wide association studies show that TIMD4, the gene coding for Tim4, a phosphatidyl serine receptor present on macrophages is associated with dyslipidemia. Here we hypothesized that a population of resident ATM expressed Tim4 and was involved in the control of postprandial circulating lipid levels.

Using protected bone marrow chimeras, we identified a population of resident ATM expressing Tim4 and representing 25% of all ATM. Flow-cytometric analysis and confocal microscopy revealed that these macrophages had a high lysosomal content and that lysosomes were loaded with neutral lipids and colocalised with Tim4, suggesting that Tim4 was involved in the lipolysis of excess triglycerides in lysosomes, a process called lysosomal lipolysis leading to the release of free fatty acid (FFA) and cholesterol. To investigate the function of Tim4, we challenged lipid homeostasis in mice with overnight high fat diet feeding and blocking Tim4 with an anti-Tim4 antibody. This led to increase postprandial circulating FFA and cholesterol which was markedly reduced by blocking Tim4. This was associated with increased lysosomal activity in Tim4+ ATM, which was inhibited by anti-Tim4 treatment, suggesting that Tim4 regulated lysosomal lipolysis. Chloroquine, an inhibitor of lysosomes equally reduced postprandial cholesterol and FFA and lysosomal activity in Tim4+ ATM. Importantly, we found that injection of clodronate liposomes which efficiently depleted peritoneal and liver macrophages, two important Tim4+ macrophages compartment, but not Tim4+ ATM, did not inhibited the increase in postprandial FFA and cholesterol levels following high fat feeding. Our results thus indicate that Tim4 play a crucial role in the control of lipid trafficking following excess triglycerides through regulation of lysosomal lipolysis in ATM.

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**P.65 Effects of APOE genotype on microglial phagocytosis of synapses in human post-mortem tissue in Alzheimer’s disease**

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Alzheimer’s disease (AD) is a lethal neurodegenerative disease of the elderly, marked by the accumulation of pathological protein aggregates, synaptic degeneration, reactive gliosis, neuroinflammation, and progressive cognitive decline. Despite knowing for over 20 years that synapse loss is the best correlate to cognitive decline (DeKosky and Scheff, 1990), the mechanisms by which synapses are lost remain elusive and there are no effective treatments to halt their degeneration. Recently, the resident immune cells and primary phagocytes of the brain, microglia, have been implicated as drivers of synaptic loss in AD by aberrantly re-activating the physiological synapse pruning mechanisms found in development (Paolicelli et al., 2011) (Hong et al., 2016).
Furthermore, the epsilon 4 allele of the apolipoprotein E gene (APOE) confers the greatest genetic risk for developing sporadic AD and patients with an APOE4 allele show exacerbated synapse loss. Here, we investigated whether in human post-mortem (PM) tissue of patients with AD, microglia phagocytose pre-synaptic elements as observed in murine models of AD, and whether the APOE genotype influences this process. We hypothesize that given the increased reactivity of APOE4 microglia as well as the early synapse loss in APOE4 patients, the reduced synapse numbers in these patients is in part due to microglial-mediated phagocytosis. To address this question, we are examining PM tissue from non-demented control and AD cases of APOE3 and APOE4 backgrounds using immunohistochemistry and confocal microscopy to quantify co-localization of synaptic markers and microglia. We have found a greater amount of pre-synaptic elements inside microglia in AD cases compared to controls in two brain areas, the temporal lobe and the primary visual cortex. Whether this is a disease associated outcome or a driving force in the disease is not clear, and we are optimising a live phagocytosis assay to assess that.

P.66 β-defensin 3 enhances macrophages polarisation towards an alternative achieved phenotype

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Macrophages are the first line of host defence against pathogens, and they are able to produce inflammatory mediators and recruit other immune cells. Two well-known types of macrophages exist: the pro-inflammatory classically activated, characterized by an increase in aerobic glycolysis, and the alternative activated, characterized by enhanced oxidative phosphorylation (OXPHOS). Pro-inflammatory macrophages, induced by interferon-γ (IFN-γ) and LPS, are involved in acute inflammation, and produce inflammatory cytokines and high level of inducible nitric oxide synthase (iNOS). On the other hand, alternatively activated macrophages, induced by IL4, are mostly involved in wound repair and tissue homeostasis, produce anti-inflammatory mediators, and exhibit increased arginase 1 (Arg-1) level.

In this study we investigate the role of the antimicrobial peptide (AMP) human β-defensin (hBD3) on macrophages polarisation. β-defensins are predominantly produced by epithelial cells in response to infection, and rapidly taken up by immune cells such as macrophages. In literature, it was been shown that hBD3 has an immunomodulatory activity in response to various PAMPs (Semple et al, 2011/2015).

Here we show that hBD3 is able to suppress the cytokine response in M(LPS+ IFN-γ) in a dose and structure dependent fashion. After hBD3 treatment, M(LPS+ IFN-γ) show reduction of pro-inflammatory cell surface markers MHC-II/CD86, and also classical activated-macrophages genes iNos and Fpr2. Moreover they exhibit an increase of both CD206/CD273, recognised as cell surface markers for alternative activated macrophages, and gene expression Arg-1 and Relm-α. M(LPS+ IFN-γ) treated with hBD3 show also activation of mitochondrial OXPHOS and decrease of glycolytic metabolism.

In summary, our data suggest that hBD3 strongly influences macrophages polarisation, with hBD3 suppressing the pro-inflammatory phenotype of M(IFN-γ/LPS), and repolarising towards an alternative activated phenotype. The ability of hBD3 to influence macrophages from a pro-inflammatory to a repair phenotype may have therapeutic implication. In vivo experimentation is underway to determine the physiological significance of these findings.
Blood, Sex, Guts and Ageing

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Immunosenescence and chronic inflammation are powerful drivers of many age-related diseases. The sexes show various differences in this inexorable system’s decline. Using the genetically malleable Drosophila melanogaster as a model, we have previously demonstrated that aged flies exhibit profound sex differences in inflammatory gut pathologies, response to oral infection and in systemic, humoral immunity. Drosophila also display dimorphic lifespans, with females outliving males, thereby mirroring sex differences in human longevity. We’re interested in understanding the role for both tissue-autonomous and systemic regulation of sexual identity within the tripartite immune system of Drosophila. We’re exploring how this regulates the ability to combat pathogens, and the propensity to develop auto-inflammation, over age. Using induced infections, we have observed a sexual dimorphism shrinkage in resistance to Providencia rettgeri as a consequence of ageing. Through genetic manipulation of sex, analysis of ageing tissue inflammation, and lifespan studies, we are elucidating the interplay between sex, immunity and ageing.


Investigating microglial lysosome function and the role of cystatin F in inflammation and neurodegenerative disease

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Inflammation is proposed to play a key role in neurodegeneration. Microglia, the major resident immune cells of the CNS, become activated in many disease paradigms. Recent studies have demonstrated that a particular type of activated microglia emerge in several models of neurodegenerative disease. We currently do not understand exactly how these disease-associated microglia (DAM) affect neurodegeneration. Among the most highly enriched gene sets in the DAM cluster are lysosomal genes, in particular Cst7 (encoding cystatin F (CF)), a lysosomal protease inhibitor. Here we aim to investigate what effect CF has on microglial function in the context of inflammation and neurodegeneration.
First we demonstrated using the specific probes Gly-Phe-AMC (Cathepsin C) or Z-Phe-Arg-AMC (Cathepsin L) that cathepsins are constitutively active in immune cells and that cathepsin activity is increased in Cst7/- cells. Next we discovered that inhibitors of cathepsin activity do not effect phagocytosis of pHrodo-tagged S.aureus bioparticles. We also demonstrated that phagocytosis of S.aureus bioparticles by BMDMs or primary microglia isolated from Cst7/- mice does not differ from WT suggesting Cst7 does not regulate phagocytosis directly. Finally, we showed that Cst7 deficiency alters neither the response of BMDMs to broad inflammatory stimuli nor the activation of the NLRP3 inflammasome or basal autophagy.

Changes in lysosomal gene expression are a key component of the microglial response to neurodegeneration. Here we have developed new tools to monitor lysosomal function in the form of cathepsin activity which can be used to interrogate the role of cystatin F in the context of neurodegenerative disease. Future studies will aim to investigate whether CF has a disease-modifying role in mouse models of neurodegenerative disease and inflammation.

P.69 Sex Differences in Drosophila melanogaster Immunity
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Sex differences in immunity exist across the animal kingdom. These dimorphisms are either cell autonomous, gonadal hormone derived, or a combination of both. It has been shown that males are often more susceptible to many infections, while females are more prone to have autoimmune disorders. Interestingly, these sex differences are still seen in insects, which lack sex hormones. Drosophila melanogaster is an excellent model organism, renowned for its well established genetic tractability. The tripartite immune repertoire of D. melanogaster is comprised of fat bodies, hemocytes and epithelial barriers such as the gut and cuticle. These correspond to mammalian liver, innate immune macrophages and gut and skin barrier defences, respectively. Furthermore, the D. melanogaster genome is fully mapped, with conservation between fly and human genes, and there are shared defense reactions with other animal phyla. As such, D. melanogaster offers a pliable model for mammalian immunity.

Using flow cytometry and microscopy, we have seen differences in the rates of phagocytosis between male and female larvae. We are also investigating differing rates of chemotaxis in male and female pupae using confocal microscopy. We will further investigate these differences in cellular immunity, with regards to immunocompetence, utilizing live pathogens, prioritising those that induce dimorphic survival. In addition, we will produce D. melanogaster with sex-switched hemocytes, resulting in males with feminized hemocytes and vice versa. This will allow for us to further differentiate whether these dimorphisms are cell autonomous or external signaling derived.
P.70 Mechanisms underlying chronic inflammation in models of impaired wound healing

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Chronic inflammation is a hallmark of impaired healing in a plethora of tissues, including skin, and is associated with aging and diseases such as diabetes. Diabetic chronic skin wounds are characterised by excessive myeloid cells that display an aberrant phenotype, and fail to resolve inflammatory responses in a timely manner, thus impeding wound healing. We found that the aberrant myeloid cell phenotype is partially mediated by stable intrinsic changes induced during hematopoietic development. We have investigated how these intrinsic changes to myeloid cells contribute to chronic inflammation and we have identified specific signaling pathways and genes that can be manipulated to ameliorate chronic inflammation in diabetic wounds. We have also identified similarities between chronic inflammation induced by diabetes and that induced by ageing. Mechanisms underlying these changes and potential therapeutic targets in chronic wounds and other chronic inflammatory diseases will be discussed.

P.71 Novel molecular mechanisms of small vessel disease – insights from rare inflammatory disease

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Cerebral small vessel disease is a major cause of dementia and stroke, yet the pathophysiological basis of SVD remains poorly understood and there are no treatments.

We have recently demonstrated a causal association between excessive type I IFN and microvascular dysfunction. Heterogeneous pathological abnormalities are observed in human microvessels that have been exposed to excessive circulating type I IFN; this finding is relevant to a spectrum of human diseases involving overactivation of the type I IFN response, e.g. lupus. A mouse model of brain intrinsic IFN-α overexpression shows comparable pathology, which can be rescued using endothelial-specific type I IFN receptor knock-out, demonstrating that endothelial cells are a target for IFN-mediated toxicity. Ongoing work using clinical samples and novel transgenic models, is aiming to understand how endothelial cells respond to systemic type I IFN, and how this can lead to microvascular dysfunction and neurological disease, such as neurolupus.

As well as responding to systemic inflammation, endothelial cells can also activate cell-intrinsic immune responses, however the pathways involved and the wider effects on associated cell of the neurovascular unit are not understood. Monogenic cerebral small vessel diseases can provide valuable insights into the molecular basis of microvascular dysfunction, and the role of cerebral endothelial cells in maintaining brain homeostasis.

Retinal Vasculopathy with Cerebral Leukoencephalopathy (RVCL) is a devastating adult-onset SVD which primarily affects the brain and retina, and rapidly leads to vascular dementia, blindness and death. It is caused by mutations in TREX1, a 3´-5´ exonuclease enzyme which degrades cellular nucleic acids and is a negative regulator of the type I IFN response. RVCL exhibits pathological overlap with interferon-mediated microangiopathy, indicating that there are common pathways involved. By studying the molecular mechanisms that underlie endothelial dysfunction in RVCL we aim to understand more about the role of endothelial cells in neuroinflammatory disease.
In the brain, inflammation due to infection and inflammation due to traumatic events are notoriously difficult to separate as both give rise to a similar response. Further, patients with suspected infection are often medicated or suffering from co-morbidities that can have major effects on the protein profile of the cerebrospinal fluid (CSF), further complicating analyses. In this pilot study we have examined whether we can use biomarkers in CSF to separate the two types of inflammation. 184 biomarkers were measured and compared in CSF from 17 control patients, 7 infected and 4 inflamed patients. Initial analysis showed increases in both infected and inflamed groups of IL-6, IL-10, IL-17 and IL-18 but no significant differences between the groups. We hypothesised that this was due to the breadth of underlying patient characteristics. Patients were then matched with multiple controls with respect to age, co-morbidities and medications and the difference between the controls and the infected/inflamed patients measured. This method showed that there was increased IL-12p70, CD40, and IL-17 in infected patients compared to those with inflammation in the absence of infection. The presence of a signature for infection in the CSF may allow us to monitor patients for early signs of neurological infection before it is evident as a culturable organism.

Intestinal inflammation and parasite sequestration during rodent malaria

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Many factors have been associated with a heightened risk of invasive bacterial disease and sepsis in children, including malaria. One overlap observed has been between Plasmodium falciparum (P. f.) and invasive non-Typhoidal Salmonellosis (iNTS), in which an enteric infection spreads systemically and causes serious disease. Whilst not often discussed, intestinal distress during uncomplicated P. f. malaria is common, with one study reporting up to 50% of infected Nigerian children presenting with abdominal pain, diarrhoea and vomiting which cleared up after administration of antimalarials. Despite associated pathology, the action of malaria infection on the gut has not been determined. It was hypothesised that parasitised red blood cells, which are known to sequester within tissues such as the brain during schizogony, can induce inflammation in the intestine. To test this, C57BL/6 mice were infected with recently mosquito-transmitted GFP-expressing P. chabaudi AS, a rodent malaria which undergoes synchronous schizogony. At various days post-infection (4, 7, 11 & 14) during schizogony, the entire intestinal tract was excised for analysis of inflammation and parasite sequestration using immunohistochemistry and immunofluorescence staining, as well as pathology by microscopy. Preliminary results suggest increased inflammatory pathology and IFNγ localisation in the intestine during infection; histology further confirmed the presence of parasites in the intestinal tissue, which has not been conclusively demonstrated previously. In combination with concurrent studies exploring the effect of parasite sequestration on systemic bacterial trafficking, we can begin to determine how malaria-induced inflammation promotes invasive bacterial disease in co-infected children. This study raises the question of whether malaria infection provides a permissive environment for invasive bacteraemia and sepsis in children – as opposed to NTS being actively ‘invasive’.
The three cytokines IL-1β, IL-18, and IL-1α share related but distinct secretory routes

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The interleukin (IL)-1-family cytokines potently regulate inflammation, with the majority of the IL-1 family proteins being secreted from immune cells via unconventional pathways. In many cases, the secretion of IL-1 cytokines appears to be coupled to cell death. Here, we studied the secretion of the three best characterized members of the IL-1 super-family, IL-1α, IL-1β, and IL-18.

It is known that IL-1β and IL-18 are cleaved by caspase-1 during inflammasome activation, which indirectly influences the calpain dependent processing of IL-1α. A range of conditions and cell types were used to study the secretion of these cytokines. Inflammasome activation was studied in murine bone marrow-derived and peritoneal macrophages, and human monocyte derived macrophages. Alternative mechanisms of IL-1α release were also studied in HeLa cells and mouse embryonic fibroblasts.

We found that upon inflammasome activation IL-1β and IL-18 shared a common secretory pathway that depended on membrane permeability, and that operated in the absence of cell lysis. We also investigated the mechanism of ASC speck release; specks are formed upon inflammasome activation and have pro-inflammatory effects when released. Whilst the release of ASC monomers was dependent on membrane permeability, the release of ASC specks was dependent on cell lysis.

The release of mature IL-1α was also dissociated from cell lysis, but independent of the effects of the membrane stabilizing agent punicalagin, which inhibited both IL-1β and IL-18 release. Finally, we further characterized the mechanism of IL-1α release showing that it is cleaved by both calpain-1 and calpain-2, and that it can also be dissociated from cell lysis in a cellular senescence model. Taken together, our results illustrate that in addition to their role as danger signals released from dead cells, IL-1 family cytokines can be secreted in the absence of cell death.

3-Hydroxykynurenine is a key mediator of critical illness and recovery in experimental acute pancreatitis

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Kynurenine monooxygenase (KMO) regulates the systemic inflammatory response during critical illness caused by acute pancreatitis (AP), but the mechanisms that link metabolism through KMO and systemic inflammation have eluded discovery, until now. Here, we show that the KMO product 3-hydroxykynurenine primes innate immune activation through upregulation of inflammatory signalling pathways and arachidonic acid metabolism to exacerbate systemic inflammation and critical illness during experimental AP. We uncover a tissue-compartmentalised role for KMO, where mice generated to lack Kmo solely in hepatocytes showed elevated plasma 3-hydroxykynurenine
levels, reduced $^{13}$C-$\Delta^1$-3-hydroxykynurenine tracer clearance, and transcriptomic alterations in key innate immunity pathways in liver tissue, specifically modulating expression of canonical toll-like receptor pathway signalling genes, including Myd88, RIP2 kinase, MAP kinases and NFκB. $\Delta^1$-hydroxykynurenine synergises with interleukin-1b to cause cellular apoptosis. Critically, mice with elevated $\Delta^1$-hydroxykynurenine succumb fatally earlier and more readily to experimental AP, and reducing $\Delta^1$-hydroxykynurenine to undetectable levels through systemic blockade using a highly-selective KMO inhibitor rescues the phenotype, protecting against critical illness and early excess mortality in experimental AP. Together, our findings establish the KMO product $\Delta^1$-hydroxykynurenine as a modulator of innate immunity that exhibits a complex interaction with inflammatory cytokines during critical illness to cause excess morbidity and death from multiple organ failure that can be rescued by systemic KMO blockade.

P.76 SLC5A12-mediated lactate influx into human CD4+ T cells at the inflamed site causes PKM2/STAT3- and fatty acid synthesis-mediated IL17 expression and tissue retention

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The tissue microenvironment is a key determinant of pathology in both inflammatory disease and cancer. Accumulation of lactate in the tumour microenvironment, largely due to the accelerated metabolism of cancer cells avidly consuming most of the scarcely available nutrients, has been shown to skew the local immune response towards immune suppression favouring tumour growth. Immune cells that infiltrate the tissue microenvironments in inflammatory disorders find similarly harsh conditions, including scarce nutrients and high levels of lactate and other metabolites. Yet opposite to the tumour microenvironment, the inflamed tissue features high levels of inflammatory cytokines with impairment of Treg responses, contributing to the exacerbation of the chronic inflammatory disease process. Indeed, in inflammatory disorders lactate is an amplifier of the inflammatory response.

Here, we explored the response of CD4+ T cells to lactate in the context of inflammation. We show that lactate accumulation at the inflamed tissue contributes, together with activating and inflammatory stimuli, to the up-regulation of the sodium-coupled lactate transporter SLC5A12 on human CD4+ T cells. SLC5A12-mediated lactate influx into human CD4+ T cells induces a plastic reshaping of their effector phenotype at the site of inflammation, resulting in increased IL17 production via nuclear PKM2/STAT3 signalling and enhanced fatty acid synthesis. It also leads to increased CD4+ T cell retention at the inflamed tissue as a consequence of reduced glycolysis and enhanced fatty acid synthesis. Furthermore, we show that lactate/SLC5A12-induced metabolic reprogramming in CD4+ T cells is a distinctive mechanism of the RA synovitis subset characterized by lymphocyte infiltration in the arthritic pannus. Finally, antibody-mediated blockade of SLC5A12 ameliorates the clinical scores in a murine model of CD4+ T cell-driven arthritis.
P.77 Investigating the Genetic Causes of Neurodevelopmental Disorders

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Neurodevelopmental disorders are an important area within the scope of medicine as for most of them there is no known treatment. Thus, constant research is currently undertaken in order to disseminate more information about several neurodevelopmental disorders such as Rett Syndrome and Autism Spectrum Disorder. Specifically, extensive research has been conducted for the genetic causes of these neurodevelopmental disorders and here we investigated further several features of these genetic causes. MeCP2, CDKL5 and FOXG1 genes are the most common genes that have been found to cause several Rett Syndrome phenotypes but according to most recent studies, there are 69 genes that are involved in Rett Syndrome.

This is a computational study, using bioinformatics to perform an analytic investigation on the gene network interactions and also compare the size of those genes which are involved in Rett Syndrome and other Rett-like neurodevelopmental disorders. Using the publicly available data, the analysis was done by developing new programs and adjust them for the needs of the study. In other words, we developed a package for common sequence analysis which is able to manage the large datasets efficiently. Our findings indicate that the genes that are involved in Rett Syndrome and in Rett-like phenotypes are exclusively “long” genes. In addition, functional analysis was performed to those genes and investigated their network interactions. The purpose of the study was to gain a better understanding of the genetic causes of neurodevelopmental disorders and offer an innovative bioinformatics program with user-friendly interface for sequence analysis.

P.78 Modulation of lung tissue repair capacity by the helminth-derived HpARI protein in type 2 lung inflammation

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Parasitic helminths are a major cause of chronic human disease, affecting more than 3 billion people worldwide. Host protection and tissue repair during most parasitic helminth infections rely upon type 2 cytokine production, and the epithelial cytokine interleukin-33 (IL-33) promotes these responses. We recently identified an IL-33-suppressive protein secreted by a mouse parasite: the Heligmosomoides polygyrus Alarmin Release Inhibitor (HpARI). HpARI binds IL-33 and nuclear DNA, tethering the cytokine within necrotic cell nuclei. Surprisingly, a truncation mutant of HpARI (CCP1/2) retains the ability to bind IL-33 and DNA, but stabilises rather than blocks the cytokine, amplifying IL-33 activity. We exploited the transient pulmonary phase of Nippostrongylus brasiliensis to study the effect of IL-33 modulation by HpARI and CCP1/2 during the recovery phase after helminth-induced lung damage and inflammation. By evaluating lung histology, we observed that CCP1/2 suppressed lung damage at early timepoints after N. brasiliensis infection. This reduced damage was linked to a rapid type 2 healing response, with increased BAL IL-5 and mucus production. Our next step will be to determine the mechanism involved in these observations. We believe that modulation of the IL-33 pathway using CCP1/2 could represent a novel tool to promote tissue repair in parasitic helminth infection.